ElBeltagy M, AlZoubi L, AbuAnzeh RB, Yousef M, AlAdwan Y, AlZoubi R, Salman A. The effect of Venlafaxine on the neuro-behavioral status of mice after administration of chemotherapeutic-neurotoxic agent (Cyclophosphamide). Pharmacy Practice. 2024 Oct-Dec;22(4):3039.

https://doi.org/10.18549/PharmPract.2024.4.3039

Original Research

The effect of Venlafaxine on the neuro-behavioral status of mice after administration of chemotherapeutic-neurotoxic agent (Cyclophosphamide)

Maha ElBeltagy, Lujain AlZoubi, Rand B. AbuAnzeh, Mohammed Yousef, Yara AlAdwan, Raha AlZoubi, Ahmad Salman

Received (first version): 19-Dec-2023 Accepted: 17-Jan-2024 Published online: 09-Oct-2024

Abstract

Background: Cyclophosphamide (CPH) is a cornerstone in cancer therapy and the present study explores the effect of Venlafaxine (VLX) against cognitive deficits induced by CPH in mice and its effect on neurogenesis in the hippocampus. **Methods:** Forty mice were randomly allocated to four groups: control, VLX, CPH, and CPH+VLX. The Novel Location Recognition model (NLR) test used here is a spatial variant of a two-trial object recognition task performed before and after the treatment with the drugs. **Results:** The control group, the VLX group, and the VLX and CPH group examined the object at the new location significantly more than the old one (P=0.04, P=0.05, and P=0.01, respectively). The test demonstrated a significant difference between Ki-67-positive cell numbers across the four groups ($p < 0.0001$). There was a significant decrease in the total number of Ki67-positive cells in the CPH group compared to the control group ($p < 0.001$). Conversely, the combined CPH and VLX group showed increased hippocampal proliferation compared to the CPH group ($p < 0.001$). There was a significant difference between GPX-1 positively stained cell numbers between control and CPH as well as control and CPH+VLX treated cells ($p < 0.001$) for both. **Conclusion:** our results show that CPH treatment caused cognitive deficits which were associated with a reduction in cell proliferation in dentate gyrus. These deficits were reduced when VLX was administered along with CPH.

Keywords: cyclophosphamide; venlafaxine; cognition; memory

INTRODUCTION

Cyclophosphamide (CPH) is considered a cornerstone in cancer therapy, whether singularly or combined with other chemotherapeutic agents.[1] CPH is used in the treatment of malignant lymphomas, breast cancer, disseminated neuroblastomas, retinoblastoma, and ovarian adenocarcinomas. Additionally, it has an immunosuppressive effect as multiple studies established it in the treatment of autoimmune diseases such as multiple sclerosis and as a pretransplant drug to prevent transplant rejection and graft vs. host complications.[2]

CPH is a type of nitrogen mustard drug that performs its effects through the alkylation of DNA.[2] by which massive cellular degeneration occurs[3] and oxidative stress is triggered in the brain.[2] Studies showed that CPH significantly induced cognitive and psychological impairments and anxiety-like behavior without causing any change in locomotor activity, specifically the hippocampal-dependent memory task.[4] through dystrophic and apoptotic changes in the hippocampal neurocyte with a marked reduction in the neurocyte cell layer thickness, thus affecting hippocampal neurogenesis that is particularly involved in learning and memory tasks.[5,6] It also reduces the number of proliferating cells in the subgranular zone of the hippocampus.[5]

Other common adverse effects reported in several studies and clinical trials include nausea and vomiting, hemorrhagic cystitis, amenorrhea, myelosuppression, and alopecia.[6] Higher doses of CPH significantly increase the incidence of these side effects and mortality.[7]

Venlafaxine (VLX) is one of the widely used antidepressants due to its relatively safe profile and its positive effects on cognition and memory impairment.[8] The serotonin-norepinephrine-dopamine reuptake inhibitor (SNDRI) VLX has been addressed to elevate monoamine levels in several brain areas related to anxiety, depression, and memory.[9] Studies found that VLX carries neuroprotective effects via its anti-inflammatory effects.[10] Animal studies have confirmed these results and showed that antidepressants can increase working and spatial memory.[11]

Maha ELBELTAGY. Department of Anatomy and Histology, School of Medicine, the University of Jordan, Faculty of Medicine, Department of Anatomy and Embryology, Menoufia University, Egypt.
Lujain ALZOUBI*. Faculty of Medicine, the University of Jordan, Amman, 11942, Jordan. lujainalzoubi2000@gmail.com
Rand B. ABUANZEH. Faculty of Medicine, the University of Jordan, Amman, 11942, Jordan.
Mohammed YOUSEF. Faculty of Medicine, the University of Jordan, Amman, 11942, Jordan.
Yara ALADWAN. Faculty of Medicine, the University of Jordan, Amman, 11942, Jordan.
Raha ALZOUBI. Faculty of Medicine, the University of Jordan, Amman, 11942, Jordan.
Ahmad SALMAN. Department of Anatomy and Histology, School of Medicine, the University of Jordan, Faculty of Medicine, Department of Anatomy and Embryology, Menoufia University, Egypt.

www.pharmacypractice.org (eISSN: 1886-3655 ISSN: 1885-642X)

https://doi.org/10.18549/PharmPract.2024.4.3039

The present study explores the protective effect of VLX against cognitive deficits induced by CPH chemotherapy in mice and examines its effect on neurogenesis in the hippocampus. Both chemotherapy and antidepressants may be altering hippocampal function which may provide a deeper understanding of the mechanism by which these drugs improve cognition during cancer treatment.

MATERIALS AND METHODS

Ethics Statement

All experiments and animal care were in accordance with The University of Jordan regulations and with local ethical committee approval.

Animals and Drug Administration

We used forty male mice (25-37 g) that were randomly allocated to four groups, each containing ten mice: control, VLX, CPH, and VLX and CPH. Animals were allowed to habituate for a period of 2 weeks before starting treatment and housed in groups of five under standard conditions of 12 hr light–12 hr dark cycle (from 8.30 a.m. to 8.30 p.m.) with free access to food and water. Behavioral testing of all animals was carried out between 8.30 a.m. and 2 p.m.

Mice in the VLX and mix groups were administered 14 intraperitoneal (i.p) 20 mg/kg doses every day (34). Mice in both the CPH and mix groups were administered 4 i.p. 50 mg/kg doses of CPH every three days (35). Drug administration duration was slightly modified for both drugs to reduce toxicity. Mice in the control group were given 4 i.p doses of 0.9% sterile saline, one injection every three days.

Behavioral testing

The Novel Location Recognition model (NLR) task was implemented to assess spatial working memory since it depends on animals' natural preference for novelty rather than positive and negative reinforcement.[12] Given its dependence on the integrity of the dentate gyrus, this task is also regarded as hippocampus-dependent.

The NLR test which is a spatial variant of a two-trial object recognition task adapted from Dix and Aggleton.[13] The apparatus was composed of an arena (a semi-transparent Perspex box, dimensions; 49 cm wide × 66 cm long × 40 cm high) and two toys (replicas, 15 cm high, 7 cm diameter). Arenas and toys (objects) were cleaned with 20% ethanol prior to each experiment and between trials to remove any remaining olfactory clues. A black square card was on the wall of the room throughout experiments to provide remarkable clues for spatial orientation.

This was modified from a previous protocol.[13] and was recorded by video camcorder as done previously in our laboratory.[14] The experiment apparatus consisted of plastic boxes (49 × 66 × 40 cm). The procedure consisted of habituating the animals for 1 hour in the box on the day prior to testing. The next day, two identical objects were put in separate locations in the box and the animals were allowed 3 min to explore (Familiarization trial). Animals then were returned to their home cage for 5 min (inter-trial interval) in which the box was cleaned by 20% ethanol. For the choice trial, the animals were put back to the box for 3 min where one object was still in its original position (familiar location) while the other object was moved to a new position (novel location).

Exploration of the object was scored when the animal sniffed, licked, chewed, or directed its nose at a distance ≤1 cm from the object.[14] Data were converted to preference indices (PI) which indicates the time spent exploring the novel object minus the time spent exploring the familiar object divided by total exploration time.[13,15,16] Exploration times of both objects and trials were recorded blind twice and averaged using a stopwatch from digitized recordings, so no observer was in the room during the trials.

Histology and Immunohistochemistry

The day after behavioral testing was completed, the rats were put down by rapid stunning and cervical dislocation. Their brains were extracted, trimmed, and fixed in 3% glutaraldehyde overnight. The next day the brains were sectioned using a Leica vibrating microtome (LEICA RM2235 Microtome, Leica Biosystems, Germany). The 4 um sections were placed onto positively charged slides for routine staining with hematoxylin and eosin and for Ki67 and Glutathione Peroxidase 1 (GPX1) immunohistochemical analysis. The tissues were dewaxed with xylene and rehydrated through a series of graded ethanols. To retrieve the Ki67 epitopes, the samples were autoclaved in 0.01 M sodium citrate, pH 9.0, at 95°C for 60 minutes in Tris/EDTA solution and then were heated in a microwave oven (800 W) for 5 minutes.[16] For GPX1, the paraffin sections were heated in a 95°C bath for 25 minutes in a sodium citrate solution (0.01 mM, pH 6.0).

After rinsing with deionized water, paraffin sections were treated with 3% hydrogen peroxide for 10 minutes at room temperature, then washed thoroughly in PBS (0.1 M, pH 7.4). Non-specific binding of immunoglobulins was inhibited with 5% bovine serum albumin (Atlas Medical, Germany) in PBS for 60 minutes at room temperature. After the blocking step, paraffin sections were incubated with antibodies to GPX1 (rabbit polyclonal, dilution of 1:750, PA5-95206, Invitrogen, USA) and Ki67 (mouse monoclonal, dilution of 1:100, ab279653, Abcam, Cambridge, UK) for one night at 4°C. Both Ki67 and GPX1 antibodies were diluted in PBS containing 0.2% Tween 20 (Tween 20 detergent, CAS 9005-64-5, Sigma Aldrich, St. Louis, MO, USA). Afterwards, paraffin sections were washed twice with PBS for 5 minutes and incubated with the complement reagent (ab236466, Abcam, UK) for 10 minutes at room temperature. Later, paraffin sections were rinsed in PBS for 5 minutes and incubated (ab236466, Abcam, UK) for 15 minutes at room temperature. The paraffin sections were first washed twice with PBS, then they were incubated with DAB for 6 minutes at room temperature, which resulted in cooler development. The paraffin sections were stained with hematoxylin for 5 minutes. Ultimately, the slides were dried with an ascending ethanol series, then cleared with fresh xylene, and coverslipped with DPX mounting media.

https://doi.org/10.18549/PharmPract.2024.4.3039

The appropriate positive and negative control slides were present in every staining run and were stored as part of the quality control system. For Ki67 and GPX1, rat intestinal epithelium tissue sections were used as positive controls. Negative controls were conducted by omitting the primary antibody and replacing it with PBS. The dark brown cytoplasmic pattern is positive for GPX1, while the dark brown nuclear pattern is positive for Ki67.

Statistical analysis

Statistical analysis was undertaken and graphs were created using GraphPad Prism 4.0. $P < 0.05$ was regarded as significant. Student's paired t-tests were used to compare the exploration times for mice in each group in the NLR task choice trials. A one-way ANOVA with Bonferroni's post-test was used to compare the number of Ki67and GPX-1-positive proliferating cells and discrimination indices between groups, and a two-way ANOVA with Bonferroni's post-test was used to compare the replicate means of the mice's weights over the injection period between all groups.

RESULTS

Effect of different treatments on weights of animals

Animals were weighed daily (Figure1). Although animals on CPH showed weight gain after the first dose, they lost weight after the third one but most importantly they were still significantly below control weights. Surprisingly, animals on both VLX and CPH showed a similar pattern of weight change to that of animals on CPH during the administration of drugs but the addition of VLX was associated with lesser weights ($P < 0.0001$).

Behavioral effects of treatments

In separate experiments, the behavioral effects of I.P CPH were tested using the NLR test. The NLR test measures interactions with objects either in familiar or novel locations within a test arena. During the familiarization trial, when animals explored two identical objects, both vehicle and drug-treated groups showed no preference for either object or the total exploration time (Figure 2).

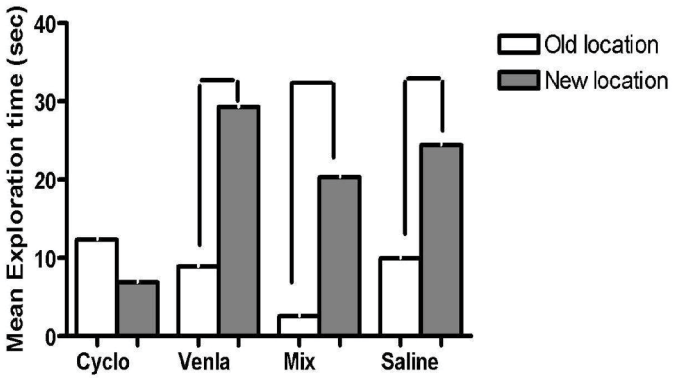


Figure 2. The Novel Location Recognition model (NLR) test

Normal saline-injected controls and mice that were treated with VLX, and the mice that received both VLX and CPH simultaneously explored the object at the new location significantly more than the old one (P=0.04, P=0.05 and P=0.01, respectively) (Figure2) On the other hand, the mice which received CPH by i.p. injection failed to differentiate between the two locations (P=0.1365).

Further analysis using the PI was done to compare the four groups (Figure 3). Comparing the PI between the control and the CPH-treated group demonstrated a significant decrease after treatment with CPH ($P < 0.05$). Comparing the PI of animals treated with CPH on its own and that of animals simultaneously given VLX and CPH showed that co-administration of VLX significantly improved preference to the new location compared with animals on CPH alone ($P < 0.001$). These findings indicate that animals receiving CPH show deficits in spatial memory which are improved by simultaneous administration of VLX.

Effect of treatments on proliferating cell counts

Immunostaining with Ki-67 was carried out to quantify the number of dividing cells in the SGZ of the dentate gyrus (Figure 4). The analysis was performed using a one-way ANOVA with Bonferroni's post-test to compare replicate means on

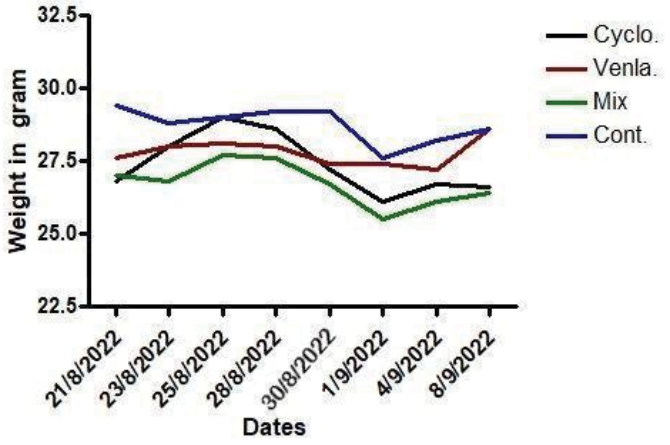


Figure 1. Average Weights of the Mice over the Experiment

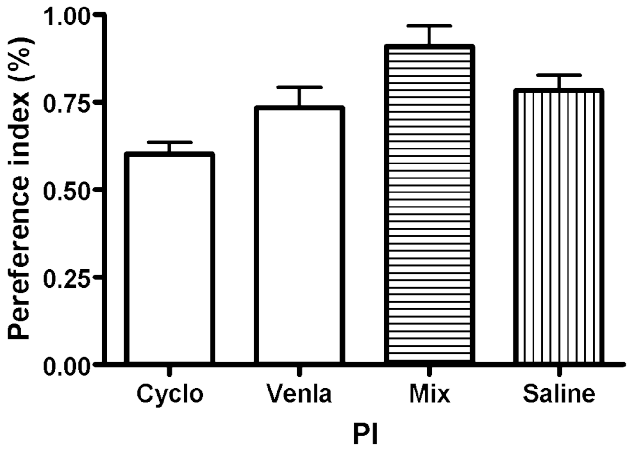


Figure 3. Preference Index (PI) of the Groups

https://doi.org/10.18549/PharmPract.2024.4.3039

a GraphPad Prism 5. The test demonstrated a significant difference between Ki-67-positive cell numbers across the four groups ($p < 0.0001$). There was a significant decrease in the total number of Ki67-positive cells in the CPH group compared to the control group ($p < 0.001$), as CPH treatment impaired hippocampal proliferation compared to saline. Conversely, the combined CPH and VLX group showed increased hippocampal proliferation compared to the CPH group ($p < 0.001$). Lastly, CPH combined with VLX had more cellular proliferation than VLX alone ($p > 0.05$)(Figure 4). VLX has not demonstrated a significant difference in proliferating cell counts when compared to all other groups but demonstrated a significant difference when compared to CPH treatment alone ($p < 0.001$) (Fig 4). In conclusion, concomitant VLX therapy was found to prevent the decrease in cellular proliferation within the SGZ caused by CPH.

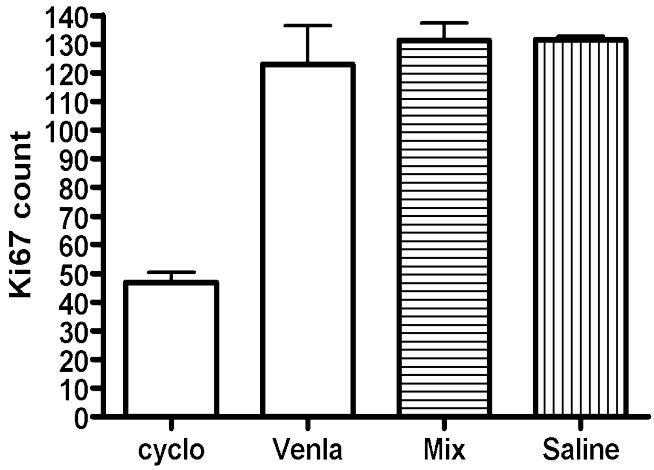


Figure 4. Mean Ki-67-Positive Cells Count

The effect of treatment on antioxidant activity

Immunostaining with GPX1 was done to assess the antioxidant activity of different treatments. There was a significant difference between positively stained cell numbers between control and CPH as well as control and CPH+ VLX treated cells ($p < 0.001$) for both. As for control versus VLX-treated cells, there was also a significant difference in positively stained cells as shown by ($p < 0.001$)(Figure5). When comparing cells treated with CPH and those with VLX, there is a significant difference ($p < 0.05$) with much fewer proliferating cells in animals treated with CPH (Figure5). In conclusion, VLX therapy with CPH was found to promote the antioxidant activity of GPX1 in addition to promoting cellular proliferation.

Representative images of Ki67 and GPX1 positive proliferating cells within the dentate gyrus in all four groups are shown in (Figure 6) and (Figure 7), respectively.

DISCUSSION

Cognitive changes -collectively termed “chemobrain” or “chemophog”- are known to be one of the major concerns among cancer survivors. Distraction, forgetfulness, and

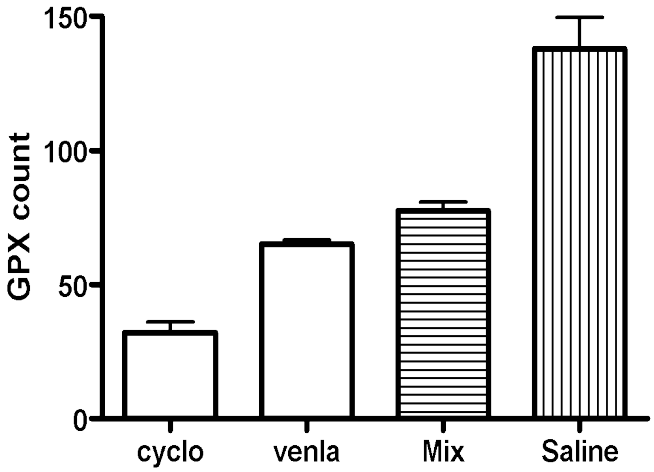
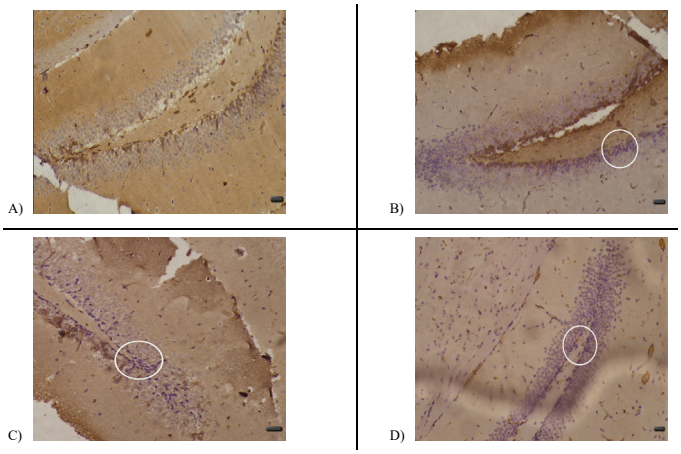
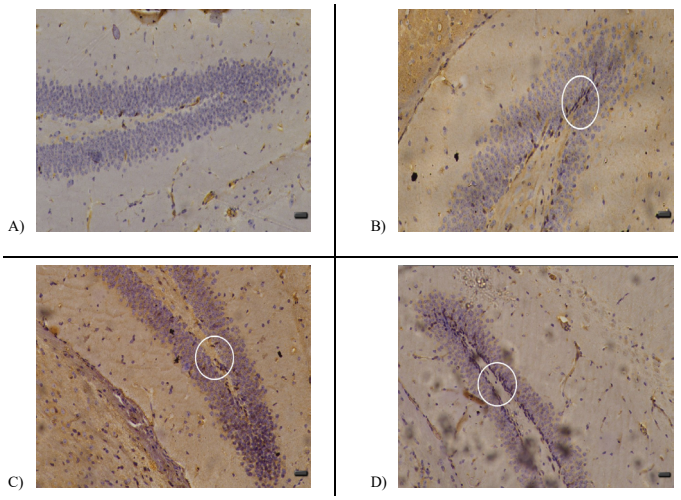


Figure 5. Mean GPX1-Positive Cells Count



Representative image of Ki67 positive proliferating cells within the dentate gyrus in A) CPH group, B) VLX group, C) MIX group, and D) control group

Figure 6. Positive Cell Proliferation for Ki-67 Marker



Representative image of GPX1 positive proliferating cells within the dentate gyrus in A) CPH group, B) VLX group, C) MIX group, and D) control group

Figure 7. Positive Cell Proliferation for GPX1 Marker

https://doi.org/10.18549/PharmPract.2024.4.3039

difficulties with attention, multitasking, and word finding were mainly what cancer survivors reported when they were asked about post-chemotherapy cognitive changes.[17] As a consequence, the patients may develop mental disorders such as anxiety and depression.[18]

The volume of the prefrontal cortex including the middle frontal gyrus, superior frontal gyrus, and frontal poles was found to be decreased on brain MRI of cancer patients,[19] along with changes in the integrity of the white matter. [20] Similarly, chemotherapy decreased the speed and distinctiveness of stimulus encoding in working memory but spared the mechanisms of perceptual encoding in EEG studies.[21,22]

In the present study, we used a mice model to explore the impact of CPH on cognitive behavior and cell proliferation, with a specific focus on its crucial role in hippocampal neurogenesis. Additionally, we sought to ascertain whether pretreatment with the antidepressant VLX could offer protection against the detrimental behavioral and cellular effects associated with CPH, including oxidative stress and neuronal damage. To assess hippocampus-dependent spatial memory, we employed the NOL test which is a well-established method.[16] The results of our study revealed that CPH-treated animals showed no significant difference in the time spent on the two objects. In contrast, animals treated with saline or VLX could readily discriminate between the novel and familiar locations of objects and spent significantly more time on the objects in the novel location. This form of discrimination necessitates the proper functioning of the dentate gyrus which diminishes when there is a reduction in hippocampal neurogenesis.[16] on the other hand, VLX was shown alongside wheel running exercise to prevent deleterious consequences of restraint stress in rats, including anxiety/depression-like behaviors and memory impairment that occur after the stress especially learned fear by upregulating brain-derived neurotrophic factor (BDNF) expression in the hippocampus.[23] VLX has also reversed the stress-induced decrease in neurogenesis and N-methyl-D-aspartate (NMDA) receptor 2B protein in the hippocampus.[24] Consequently, Animals receiving both i.p. injections of CPH and VLX show an improvement in their discrimination between familiar and novel object locations and could significantly discriminate between these positions. Moreover, this was confirmed by calculating the PI, which demonstrated that animals spent much more time on the object in the novel location than would be predicted by chance. However, the CPH-treated animals showed a significant decrease in selecting the object in the novel location (Figure2). This behavioral data aligns with previous research findings which revealed that CPH can cause cognitive deficits and impair spatial memory in animal models.[3-6]

Regarding the effect of CPH on spatial memory, one possible cause could be the disruption of adult hippocampal neurogenesis. The production of new neurons in the subgranular zone (SGZ) for incorporation into the dentate gyrus, is a well-characterized phenomenon in all mammals including humans.[26] Reductions in cell proliferation in the SGZ by pharmacological, environmental, or genetic means are associated with deficits in memory.[15,27] Some reports have found that antidepressants increase the proliferation of cells in the SGZ[28]while others have failed to find this effect.[29,30] In the present study, we showed that CPH significantly decreased the number of proliferating cells in the SGZ of the dentate gyrus when compared to the controls using immunohistochemistry for the proliferative markers Ki67 and GPX. The results of this study also showed that VLX-treated animals had a higher number of proliferating cells in the SGZ when compared with mice treated with CPH only. So when VLX was administered concomitantly with CPH, it was able to limit the decrease in cognition and cellular proliferation found in animals treated with CPH alone.

Understanding the effects of chemotherapy on patients is complicated by other aspects of the disease and treatment and cancer patients can exhibit cognitive impairments prior to treatment.[31] The present study shows that CPH can produce cognitive decline in the behavioral test and this is associated with a decrease in proliferation in the neurogenic region of the hippocampus. These results are in line with animal studies of other chemotherapy agents which have found cognitive declines after treatment.[14,32,33]

The effect of VLX on weight is still controversial, few studies conducted on both animals and humans reported a decrease in weight among using VLX.[25,38] Whereas other studies reported its association with weight gain.[39] Weight loss was seen in both of the groups that were injected with CPH, and such a finding is in line with the literature, as CPH was found to cause rapid and significant weight loss and/or inhibition of weight gain.[40] The damage to liver cells, which have important roles in digestion and metabolism, was suggested as one of the mechanisms by which CPH affects weight. Additionally, the impairment of the immune system that CPH can cause, may also have a role in weight loss.[41]

The hippocampal dentate gyrus, a critical brain region implicated in learning and memory functions, displays a high susceptibility to oxidative stress induced by reactive oxygen species (ROS), as demonstrated in prior research.[36] This oxidative stress poses a substantial risk to cells, resulting in detrimental consequences such as damage and the eventual demise of crucial cellular constituents, including lipids, proteins, and DNA.[37] Indeed, it is worth emphasizing that the oxidative injury inflicted upon neuronal components serves as the fundamental molecular underpinning of both neurodegenerative processes and the natural aging of the brain. CPH exerts its pharmacological effects primarily through a process known as DNA alkylation.[2] This intricate molecular mechanism instigates a cascade of events within the cellular milieu, resulting in extensive cellular degeneration and the initiation of oxidative stress within the cerebral context.[4]

In conclusion, our results show that CPH treatment caused cognitive deficits which were associated with a reduction in cell proliferation in the SGZ of the dentate gyrus. These deficits were reduced when VLX was administered throughout the CPH treatment period. Extensive research endeavors have unveiled the profound impact of CPH on cognitive and

psychological domains, with a pronounced emphasis on its deleterious effects on hippocampal-dependent memory tasks.[5] These cognitive impairments can be attributed, in part, to the dystrophic and apoptotic transformations that hippocampal neurocytes undergo when exposed to CPH.[6]

Animals on CPH showed weight gain after the first dose and they lost weight after the third and they were significantly below control weights. Animals in the mix group showed a similar pattern of weight change to that of animals on CPH where the addition of VLX was associated with lesser weights ($P < 0.0001$).

The control group, the VLX group, and the mix group explored the object at the new location significantly more than the old one (P=0.04, P=0.05 and P=0.01, respectively). On the other hand, the CPH group failed to differentiate between the two locations (P=0.1365).

The PI of the CPH group in comparison to the control group was significantly lesser ($P < 0.05$). The PI of CPH group in comparison to the mix group showed that co-administration of VLX significantly improved preference to the new location compared with animals on CPH alone ($P <0.001$).

Significant difference between Ki-67-positive cell numbers across the four groups ($p < 0.0001$). Moreover, there was a significant decrease in the total number of Ki67-positive cells in the CPH group compared to the control group ($p < 0.001$). The mix group showed significant increased proliferation compared to the CPH group ($p < 0.001$). Lastly, the mix group had more cellular proliferation than the VLX group ($p > 0.05$). tThe VLX group did not demonstrate a significant difference in proliferating cell counts when compared to all other groups. except when compared to the CPH group ($p < 0.001$).

There was a significant difference between positively stained cell numbers between the control group and the CPH group($p < 0.001$). Similarly, the control group and the mix group ($p < 0.001$). As for control versus VLX groups, there was also a significant difference in positively stained cells ($p < 0.001$). When comparing the CPH group and the VLX group, there is a significant difference ($p < 0.05$) with much fewer proliferating cells in animals treated with CPH.

DECLARATIONS

DATA AVAILABILITY STATEMENT: The data that support the findings of this study are available from the corresponding author upon reasonable request.

FUNDING: The research leading to these results received funding from the Deanship of Academic Research, University of Jordan, under Grant Agreement No 2022-2021/16.

CONFLICTS OF INTEREST: The authors have no conflicts of interest to declare that are relevant to the content of this article.

AUTHOR ROLES: All authors contributed to the study conception and design. Drug preparation, injection and animals weighing were performed by L.A, R.AB, M.Y, Y.A and R.AL. Formal analysis was performed by M.E and A.S. The first draft of the manuscript was written by L.A, R.AB, M.Y, Y.A and R.AL. Reviewing and editing the manuscript were performed by M.E and A.S. All authors read and approved the final manuscript. Funding and resources acquisition was performed by A.S. Supervision was performed by M.E.

References

1. Lee JH, Nan A.Combination drug delivery approaches in metastatic breast cancer. Journal of drug delivery. 2012;2012:915375. https://doi.org/10.1155/2012/915375
2. Ogino MH, Tadi P. Cyclophosphamide. In StatPearls. StatPearls Publishing. (2022).
3. Oraby HA, Hassan AA, Maaty NF. Effects of cyclophosphamide on transcription of SOD1 mRNA and GPX1 mRNA in mice liver and brain tissue. J Appl Biosci. 2010;29:1736-42.
4. Bhatia AL, Manda K, Patni S, Sharma AL. Prophylactic action of linseed (Linum usitatissimum) oil against cyclophosphamide-induced oxidative stress in mouse brain. Journal of medicinal food. 2006;9(2):261-4. https://doi.org/10.1089/jmf.2006.9.261
5. Kitamura Y, Kanemoto E, Sugimoto M, Machida A, Nakamura Y, Naito N, et al. Influence of nicotine on doxorubicin and cyclophosphamide combination treatment-induced spatial cognitive impairment and anxiety-like behavior in rats. Naunyn-Schmiedeberg's archives of pharmacology.2017;390(4):369-78. https://doi.org/10.1007/s00210-016-1338-z
6. Ibrahim HM, Zommara MA, Elnaggar ME. Ameliorating effect of selenium nanoparticles on cyclophosphamide-induced hippocampal neurotoxicity in male rats: light, electron microscopic and immunohistochemical study. Folia morphologica. 2021;80(4):806-19. https://doi.org/10.5603/fm.a2020.0117
7. Korkmaz A, Topal T, Oter S. Pathophysiological aspects of cyclophosphamide and ifosfamide induced hemorrhagic cystitis; implication of reactive oxygen and nitrogen species as well as PARP activation. Cell biology and toxicology. 2007;23(5):303-12. https://doi.org/10.1007/s10565-006-0078-0
8. Li L, Zhang C. Venlafaxine Attenuated the Cognitive and Memory Deficit in Mice Exposed to Isoflurane Alone. Frontiers in neurology. 2021;12:591223. https://doi.org/10.3389/fneur.2021.591223
9. Dawson LA, Nguyen HQ, Geiger A. Effects of venlafaxine on extracellular concentrations of 5-HT and noradrenaline in the rat frontal cortex: augmentation via 5-HT1A receptor antagonism. Neuropharmacology. 1999;38(8):1153-63. https://doi.org/10.1016/s0028-3908(99)00052-0
10. Zepeda R, Contreras V, Pissani C, Stack K, Vargas M, Owen GI, et al. Venlafaxine treatment after endothelin-1-induced cortical

stroke modulates growth factor expression and reduces tissue damage in rats. Neuropharmacology. 2016;107:131-45. https://doi.org/10.1016/j.neuropharm.2016.03.011
11. Lapmanee S, Charoenphandhu J, Teerapornpuntakit J, Krishnamra N, Charoenphandhu N. Agomelatine, venlafaxine, and running exercise effectively prevent anxiety- and depression-like behaviors and memory impairment in restraint stressed rats. PloS one. 2017;12(11):e0187671. https://doi.org/10.1371/journal.pone.0187671
12. Berlyne DE. Novelty and curiosity as determinants of exploratory behaviour. British journal of psychology. 1950;41(1):68.
13. Dix SL, Aggleton JP. Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. Behavioural brain research. 1999;99(2):191-200. https://doi.org/10.1016/s0166-4328(98)00079-5
14. Mustafa S, Walker A, Bennett G, Wigmore PM. 5-Fluorouracil chemotherapy affects spatial working memory and newborn neurons in the adult rat hippocampus. The European journal of neuroscience.2008;28(2):323-30. https://doi.org/10.1111/j.1460-9568.2008.06325.x
15. Bruel-Jungerman E, Laroche S, Rampon C. New neurons in the dentate gyrus are involved in the expression of enhanced long-term memory following environmental enrichment. The European journal of neuroscience. 2005;21(2):513-21. https://doi.org/10.1111/j.1460-9568.2005.03875.x
16. ElBeltagy M, Mustafa S, Umka J, Lyons L, Salman A, Chur-yoe GT, et al. Fluoxetine improves the memory deficits caused by the chemotherapy agent 5-fluorouracil. Behavioural brain research. 2010;208(1):112-7.
17. Ahles TA, Root JC, Ryan EL. Cancer- and cancer treatment-associated cognitive change: an update on the state of the science. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2012;30(30):3675-86. https://doi.org/10.1200/jco.2012.43.0116
18. Simó M, Rifà-Ros X, Rodriguez-Fornells A, Bruna J. Chemobrain: a systematic review of structural and functional neuroimaging studies. Neuroscience and biobehavioral reviews. 2013;37(8):1311-21. https://doi.org/10.1016/j.neubiorev.2013.04.015
19. McDonald BC, Conroy SK, Ahles TA, West JD, Saykin AJ. Gray matter reduction associated with systemic chemotherapy for breast cancer: a prospective MRI study. Breast cancer research and treatment, 2010;123(3):819-28. https://doi.org/10.1007/s10549-010-1088-4
20. Deprez S, Amant F, Yigit R, Porke K, Verhoeven J, Van den Stock J, et al. Chemotherapy-induced structural changes in cerebral white matter and its correlation with impaired cognitive functioning in breast cancer patients. Human brain mapping2011;32(3):480-93. https://doi.org/10.1002/hbm.21033
21. Kreukels BP, Schagen SB, Ridderinkhof KR, Boogerd W, Hamburger HL, Muller MJ, et al. Effects of high-dose and conventional-dose adjuvant chemotherapy on long-term cognitive sequelae in patients with breast cancer: an electrophysiologic study. Clinical breast cancer. 2006;7(1):67-78. https://doi.org/10.3816/cbc.2006.n.015
22. Kreukels BPC, Hamburger HL, de Ruiter MB, van Dam FSAM, Ridderinkhof KR, Boogerd W, et al. ERP amplitude and latency in breast cancer survivors treated with adjuvant chemotherapy. Clinical neurophysiology: official journal of the International Federation of Clinical Neurophysiology. 2008;119(3):533-41. https://doi.org/10.1016/j.clinph.2007.11.011
23. Lapmanee S, Charoenphandhu J, Teerapornpuntakit J, Krishnamra N, Charoenphandhu N. Agomelatine, venlafaxine, and running exercise effectively prevent anxiety- and depression-like behaviors and memory impairment in restraint stressed rats. PloS one. 2017;12(11):e0187671. https://doi.org/10.1371/journal.pone.0187671
24. Zhang X, Li X, Li M, Ren J, Yun K, An Y, et al. Venlafaxine increases cell proliferation and regulates DISC1, PDE4B and NMDA receptor 2B expression in the hippocampus in chronic mild stress mice. European journal of pharmacology. 2015;755:58-65. https://doi.org/10.1016/j.ejphar.2015.02.044
25. Sasváriová M, Michálíková D, Kaprinay BT, Salvaras L, Hričáková S, Knezl V, et al. The effect of venlafaxine on blood pressure and ECG in rats fed with high-fat-fructose diet. Interdisciplinary toxicology. 2019;12(4):192-9. https://doi.org/10.2478/intox-2019-0024
26. Imayoshi I, Sakamoto M, Ohtsuka T, Kageyama R. Continuous neurogenesis in the adult brain. Development, growth & differentiation. 2009;51(3):379-86. https://doi.org/10.1111/j.1440-169x.2009.01094.x
27. Jessberger S, Clark RE, Broadbent NJ, Clemenson GD Jr, Consiglio A, Lie DC, et al. Dentate gyrus-specific knockdown of adult neurogenesis impairs spatial and object recognition memory in adult rats. Learning & memory (Cold Spring Harbor, N.Y.). 2009;16(2):147-54. https://doi.org/10.1101/lm.1172609
28. Kodama M, Fujioka T, Duman RS. Chronic olanzapine or fluoxetine administration increases cell proliferation in hippocampus and prefrontal cortex of adult rat. Biological psychiatry. 2004;56(8):570-80. https://doi.org/10.1016/j.biopsych.2004.07.008
29. Holick KA, Lee DC, Hen R, Dulawa SC. Behavioral effects of chronic fluoxetine in BALB/cJ mice do not require adult hippocampal neurogenesis or the serotonin 1A receptor. Neuropsychopharmacology: official publication of the American College of Neuropsychopharmacology. 2008;33(2):406-17. https://doi.org/10.1038/sj.npp.1301399
30. Cowen DS, Takase LF, Fornal CA, Jacobs BL. Age-dependent decline in hippocampal neurogenesis is not altered by chronic treatment with fluoxetine. Brain research. 2008;1228;14-19. https://doi.org/10.1016/j.brainres.2008.06.059
31. Wefel JS, Lenzi R, Theriault R, Buzdar AU, Cruickshank S, Meyers CA. 'Chemobrain' in breast carcinoma?: a prologue. Cancer. 2004;101(3):466-75. https://doi.org/10.1002/cncr.20393
32. Konat GW, Kraszpulski M, James I, Zhang HT, Abraham J. Cognitive dysfunction induced by chronic administration of common cancer chemotherapeutics in rats. Metabolic brain disease. 2008;23(3):325-33. https://doi.org/10.1007/s11011-008-9100-y

33. Seigers R, Schagen SB, Beerling W, Boogerd W, van Tellingen O, van Dam FS, et al. Long-lasting suppression of hippocampal cell proliferation and impaired cognitive performance by methotrexate in the rat. Behavioural brain research. 2008;186(2):168-75. https://doi.org/10.1016/j.bbr.2007.08.004
34. Abdel-Wahab B, SalamaR. Venlafaxine protects against stress-induced oxidative DNA damage in hippocampus during antidepressant testing in mice.Pharmacol Biochem Behav. 2011;100(1):59-65 https://doi.org/10.1016/j.pbb.2011.07.015
35. Janelsins M, Roscoe J, Berg M, Thompson B, Gallagher M, Morrow G, et al. IGF-1 Partially Restores Chemotherapy-Induced Reductions in Neural Cell Proliferation in Adult C57BL/6 Mice. Cancer Invest. 2010;28(5):544-53. https://doi.org/10.3109/07357900903405942
36. Gottlieb M, Leal-Campanario R, Campos-Esparza MR, Sánchez-Gómez MV, Alberdi E, Arranz A, et al. Neuroprotection by two polyphenols following excitotoxicity and experimental ischemia. Neurobiology of disease. 2006;23(2):374-86. https://doi.org/10.1016/j.nbd.2006.03.017
37. Gilgun-Sherki Y, Melamed E, Offen D. Oxidative stress induced-neurodegenerative diseases: the need for antioxidants that penetrate the blood brain barrier. Neuropharmacology. 2001;40(8):959-75. https://doi.org/10.1016/s0028-3908(01)00019-3
38. Oliveira R, Cunha G, Borges K, Bruin G, Santos-Filho E, Viana G, et al. The effect of venlafaxine on behaviour, body weight and striatal monoamine levels on sleep-deprived female rats.
39. Carvalho,A. Sharma,M. Brunoni,A. Vieta,E. Fava,G. The Safety, Tolerability and Risks Associated with the Use of Newer Generation Antidepressant Drugs: A Critical Review of the Literature. Psychother psychosom. 2016;85(5):270-88 https://doi.org/10.1159/000447034
40. Zhang Z, Pan T, Liu C, Shan X, Xu Z, Hong H, et al. Cyclophosphamide induced physiological and biochemical changes in mice with an emphasis on sensitivity analysis. Ecotoxical Environ Saf. 2021 Mar 15:211:111889. https://doi.org/10.1016/j.ecoenv.2020.111889
41. Kumamoto Y, Camporez JPG, Jurczak MJ, Shanabrough M, Horvath T, Shulman GI, et al. CD301b(+) mononuclear phagocytes maintain positive energy balance through secretion of resistin-like molecule alpha. Immunity. 2016;45(3):583-96. https://doi.org/10.1016/j.immuni.2016.08.002