

## Review Form 1.6

Journal Name:	<a href="#">Journal of Pharmaceutical Research International</a>
Manuscript Number:	Ms_JPRI_79620
Title of the Manuscript:	Synthesis And Scanning Electron Microscopy Analysis Of N-Phthaloyl GABA and Its Influence in Improving the Cognitive Performance in Sleep-Deprived Zebrafish
Type of the Article	Original Research Article

### **General guideline for Peer Review process:**

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound. To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

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**PART 1: Review Comments**

	<b>Reviewer's comment</b>	<b>Author's comment</b> (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
<p><b>Compulsory</b> REVISION comments</p>	<p>This article explored the ability of a synthetic analogue of GABA, N-phthaloyl-<math>\gamma</math>-aminobutyric acid (P-GABA), to alleviate the behavioral, histopathological and biochemical adverse effects of sleep deprivation in zebrafish (<i>Dario rerio</i>). This manuscript was badly written. Besides some methodological flaws, it contains a large number of errors and inaccuracies. I have pinpointed quite a number of them below, but an extensive linguistic revision for English morphology, vocabulary, grammar, punctuation, hyphenation, syntax and style is in order. Notwithstanding its limitations, this manuscript conveys some interesting observations and may be considered for publication, in case the Authors agree and are able to provide a major revision, conveniently addressing all the matters of concern indicated below.</p> <p><b>Article title</b> The synthesis of N-phthaloyl-GABA is not a novel achievement and should not be reported as such, much less in the article title. Furthermore, the electro-microscopic analysis was not the most appropriate way to verify the successful synthesis of the product from a chemical standpoint, which is what most counts, and therefore also this aspect does not deserve to be mentioned in the title. Please rephrase the article title as "A study of the alleviating effects of N-phthaloyl-<math>\gamma</math>-aminobutyric acid (P-GABA) on the behavioral, histopathological and biochemical adverse effects of sleep deprivation in zebrafish (<i>Dario rerio</i>)".</p> <p><b>Abstract</b> The Authors ought to report concisely in the Results section of the Abstract, in addition to the results obtained with the T-maze test and the Light/Dark test, also the results of the SOD, CAT and GPX activities, the AChE activity, the MDA level, the whole-body cortisol level and the brain levels of dopamine, serotonin and norepinephrine. This should substitute for the sentence "According to the researchers, P-GABA administration to sleep-deprived fish restores the antioxidants and lipid peroxidation products levels to normal" presently contained in page 1, lines 6-7 from the bottom.</p> <p><b>Introduction</b> - page 2, line 2: please rephrase "when it comes to animals, in the anterior hypothalamus, the suprachiasmatic nucleus (SCN) is the master clock as it regulates ..." as "the master biological clock in animals is located in the suprachiasmatic nucleus (SCN) of the anterior part of the hypothalamus, which regulates ..."; - page 2, line 6: please delete "which are autonomous and limited to 24 hours every day"; Fig. 1 with its introductory statement should be moved to the end of the Methodology section.</p> <p><b>Methodology</b> - page 3, line 3 from the bottom: rephrase "To get the required outcome, an intimate mixture ..." simply as "A mixture ..."; - page 4, line 1, subparagraph "2.2 N-Phthaloyl-GABA preparation": the Authors state "the result was recognized as previously stated [26, 27]". In regard, in reference 26 it is reported that newly synthesized P-GABA "was identified by: (a) measuring the melting point, (b) UV absorption, and IR spectroscopy; c) thin-layer chromatography of the product using n-propanol: water (7:3), thereby determining the Rf value." The Authors please state explicitly whether these analyses were performed and please use the wording "the product was identified", instead of "the result was recognized". The analytical approaches just cited seem much more appropriate from a chemical point of</p>	

	<p>view than the morphological assessment via SEM that the Authors presented in section 3.1. The Authors please also specify how was this material filtered, whether and how was it desiccated and put onto which grids for SEM examination;</p> <ul style="list-style-type: none"> <li>- page 4, line 15: please delete the statement “Furthermore, P-GABA effects are prominent in this study”;</li> <li>- page 4, paragraph 2.4: please rename the paragraph heading as “Assay of enzymatic activities and oxidative stress biomarkers”. The Authors reported having assayed the activities of superoxide dismutase (please specify whether SOD1, SOD2, SOD3 or else), catalase (CAT), glutathione peroxidase (GPX) and acetylcholinesterase (AChE), as well as the concentration of malondialdehyde (MDA) in the supernatants of brain homogenates, yet in the legend to Fig. 8 they mention “oxidative stress biomarkers in <i>Danio rerio</i> serum”. Please confirm and indicate in the legend to Fig. 8 that the assays of enzymatic activities were indeed performed on fish brain homogenates. The same applies to the legend to Fig. 9, concerning the assay of acetylcholinesterase activity. Please also indicate the EC number of each enzyme considered, according to the international Enzyme Commission Number classification;</li> <li>- page 4, line 8 from the bottom: please amend “freezing water (2-4 °C)” to “cold water (2-4 °C)”;</li> <li>- page 4, line 3 from the bottom: Add the “MDA” abbreviation in brackets after mentioning malondialdehyde;</li> <li>- page 5, line 1: please delete “According to previous reports” and, further below, delete “According to the manufacturer’s recommendations”. Also, the Author(s) please specify what do they mean with “cortisol was taken from the fish’s entire body, with six fish per sample pooled together to meet the 0.5 g weight threshold for the cortisol test”. Do they mean that cortisol was assayed on the whole-body homogenate of six fishes per each experimental group? Please provide more details as to the preparation of the homogenates, the extraction of cortisol from the homogenates and the concentration of extracts for analysis by ELISA;</li> <li>- page 5, line 7: amend “cleared” to “included” and delete “Paraffin blocks were used to implant the samples”;</li> <li>- page 5, paragraph “2.6 Histopathology”: please move this paragraph right after paragraph 2.3 and renumber the other paragraphs in the Methodology section accordingly;</li> <li>- page 5, paragraph “2.7 Neurotransmitter analysis”: please confirm and mention that all assays of neurotransmitters were performed on brain homogenates and rename this paragraph as “Assays of neurotransmitters in brain homogenates”. Also rename subparagraph 2.7.1 as “Assays of norepinephrine and dopamine”;</li> <li>- page 5, lines 19-20: delete the sentence “Adjustments to the reagent and solvent concentrations were required to achieve the necessary fluorescence yield while utilizing smaller vials”.</li> </ul> <p><b>Results</b></p> <ul style="list-style-type: none"> <li>- page 5, paragraph “3.1 Characterization of P-GABA”: please refer to my comment above to page 4, line 1 of the Methods. Add details to the text or to the legend of Fig. 3 concerning how was synthetic P-GABA filtered, whether and how was it desiccated and put onto which grids for SEM examination;</li> <li>- page 6, legend to Fig. 4: please delete “This graph shows the”;</li> <li>- page 8, paragraph “3.4 Effect on biochemical parameters”: please rename as “3.4 Effects on antioxidant enzymatic activities”. Please confirm and mention that the activities of the enzymes indicated were assayed in fish brain homogenates. Please delete the following sentences: “indicating that SOD has promising antioxidant potential in this environment” (page 8, line 8), “indicating antioxidant properties: As a bonus” (page 8, line 11). Also amend “administered” to “applied” (page 8, line 11);</li> <li>- page 8, legend to Fig. 8: Please rephrase “Oxidative stress biomarkers in <i>Danio rerio</i> serum” as “Antioxidant enzyme activities in <i>Danio rerio</i> brain homogenates”;</li> <li>- page 8, paragraph 3.5 and legend to Fig. 9: please confirm and indicate that acetylcholinesterase activity was assayed in fish brain homogenates;</li> <li>- page 9, lines 1-4: spell out “MDA” as malondialdehyde (MDA) in the paragraph</li> </ul>	
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	<p>heading and briefly mention at the beginning of the paragraph that malondialdehyde derives from the peroxidation of polyunsaturated fatty acids. Please also confirm and indicate that MDA concentration was assayed in fish brain homogenates, also in the legend to Fig. 9;</p> <ul style="list-style-type: none"> <li>- page 10, paragraph "3.7 Histopathology": please move this paragraph right after paragraph 3.3 and renumber the other paragraphs in the Results section accordingly;</li> <li>- page 10, legend to Fig. 10: amend "parameter" to "product".</li> </ul> <p><b>Discussion</b></p> <ul style="list-style-type: none"> <li>- page 12, line 2: please delete "solid";</li> <li>- page 12, line 4 from the bottom: please delete "According to researchers" and amend "restores" as "restored";</li> <li>- page 12, line 12 from the bottom: please delete "According to researchers";</li> <li>- page 13, line 17 from the bottom: please delete "When it comes to the brain";</li> <li>- page 14, line 9: amend "reposition P-GABA" to "repositioning of P-GABA".</li> </ul>	
<b>Minor</b> REVISION comments		
<b>Optional/General</b> comments		

**PART 2:**

	<b>Reviewer's comment</b>	<b>Author's comment</b> (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
<b>Are there ethical issues in this manuscript?</b>	<i>(If yes, Kindly please write down the ethical issues here in details)</i>	

**Reviewer Details:**

Name:	<b>Fabrizio Gentile</b>
Department, University & Country	<b>Università Degli Studi Del Molise, Italy</b>