Review Form 3

Journal Name:	Asian Journal of Research in Biochemistry
Manuscript Number:	**Ms_AJRB_125353**
Title of the Manuscript:	**Impact of Diphenyl Diselenide on Acute Ethanol-Induced Disruption of Antioxidant Defense and Inflammatory Gene Expression in Rats**
Type of the Article	

PART 1: Review Comments

Compulsory REVISION comments	**Reviewer's comment**	**Author's Feedback** *(Please correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)*
Please write a few sentences regarding the importance of this manuscript for the scientific community. Why do you like (or dislike) this manuscript? A minimum of 3-4 sentences may be required for this part.		
Is the title of the article suitable? (If not please suggest an alternative title)		
Is the abstract of the article comprehensive? Do you suggest the addition (or deletion) of some points in this section? Please write your suggestions here.		
Are subsections and structure of the manuscript appropriate?		
Please write a few sentences regarding the scientific correctness of this manuscript. Why do you think that this manuscript is scientifically robust and technically sound? A minimum of 3-4 sentences may be required for this part.		
Are the references sufficient and recent? If you have suggestions of additional references, please mention them in the review form. -		

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Minor REVISION comments **Is the language/English quality of the article suitable for scholarly communications?**		
Optional/General comments	Impact of Diphenyl Diselenide on Acute Ethanol-Induced Disruption of Antioxidant Defense and Inflammatory Gene Expression in Rats was reported in the manuscript. The manuscript is not well organized, and some issues should be addressed. The manuscript cannot be accepted for publication in the present form but after the major revision it can be published. Detailed comments are as follows: **Abstract…….** **Comment1:** The Author(s) mentions that DPDSe was administered orally 30 minutes before and after ethanol administration, which might suggest a two-dose regimen. Author(s) could be clarified whether the animals received both pre- and post-treatment, or if it was either pre- or post-treatment in different groups. **Comment2:** The Author(s) states, "ethanol evoked high production of lipid peroxidation" but does not quantify or elaborate on how lipid peroxidation was measured (e.g., MDA or TBARS assay). Including a brief reference to the method would strengthen this point. **Comment3:** The Author(s) mentions "reversal of ethanol-induced changes" but could benefit from elaborating on the proposed mechanism by which DPDSe exerts its effects. For instance, linking its antioxidant properties to modulation of redox-sensitive transcription factors (e.g., Nrf2) could provide a more mechanistic insight. **Comment4:** The role of NF-kB as a pro-inflammatory marker could also be expanded slightly. Author(s) might discuss how DPDSe's inhibition of NF-kB signaling could mitigate ethanol-induced inflammation. **Comment5:** Consider adding more detail on the observed changes in purinergic enzyme activity, such as the specific fold increase or percentage change in NTPDase and nucleotidase activities due to ethanol intoxication, as this is a significant finding. **Comment6:** It's implied but not explicitly stated whether there was a control group that received ethanol without DPDSe or a group that received only DPDSe. Mentioning the control groups briefly would enhance understanding of the study design. **Comment7:** Instead of "perturbation in antioxidant status," Author(s) could use "disruption of the antioxidant defense system" to convey a clearer understanding of oxidative stress. **Comment8:** The phrase "alteration in activities of purinergic enzymes" can be more specific. Consider mentioning the exact enzymes impacted earlier (such as NTPDase and 5′-nucleotidase). **Comment9:** You can further define "thiol redox system" for clarity, explaining it relates to the balance of reduced (GSH) and oxidized (GSSG) forms of thiols. Thus, revised version maintains the original findings while clarifying terminology, experimental design, and mechanisms, making the abstract more precise and reader-friendly. **Introduction……** **Comments10:** The introduction starts with the complex effects of ethanol intoxication but	

	quickly shifts focus to specific mechanisms (redox-sensitive genes and purinergic enzymes). While these topics are relevant, the transition between these mechanisms and their connection to ethanol's broader impact is somewhat abrupt. To improve coherence, consider restructuring this section by first summarizing the overall impact of ethanol on liver cells and oxidative stress, then smoothly transitioning into the molecular details. **Comments11:** The link between oxidative stress, inflammation, and purinergic enzyme activity could be made clearer. Emphasize that oxidative stress is a central mechanism of ethanol intoxication, which leads to both inflammation and changes in purinergic enzyme activity. **Comments12:** Author(s) should Clarify the Mechanisms of Action. **Comments13:** Author(s) should Discuss the Role of Antioxidants More Thoroughly **Comments14:** Author(s) should Rationale for Studying Diphenyl Diselenide (DPDSe) **Comments15:** Author(s) stated that the effects of DPDSe on acute ethanol intoxication in male Wistar rats are not well understood, it would be helpful to specify the gap in the existing literature more clearly. For instance, mention if other antioxidant compounds have been tested in this model and how DPDSe's unique properties might address limitations of these other treatments. **Comments16:** Author(s) should Provide a more direct statement on the objective of the study at the end of the introduction. Currently, the aim is mentioned briefly, but it could be framed as addressing a key gap in knowledge regarding the therapeutic potential of DPDSe for acute liver damage induced by ethanol intoxication. **Comments17:** The weight range of the rats (120–150 g) is mentioned, but the age of the animals could also be provided. Age is often an important factor in metabolic and physiological studies. Additionally, specify whether the rats were housed under controlled conditions (e.g., temperature, humidity, light/dark cycle) and whether they had ad libitum access to food and water. **Comments18:** Author(s) mention that the rats were acclimatized for two weeks, but it would be helpful to include the specific environmental conditions (e.g., temperature, humidity, light cycle) and confirm that they were fed a standard diet. This would ensure that the experimental protocol is reproducible. **In Vitro Study on Thiol Oxidation (Section 2.3)** **Comments19:** Author(s) should give More detail on assay conditions: as mention that the assay was conducted by measuring the formation of 2-nitro-5-thiobenzoic acid (TNB) at 412 nm, more details on the conditions under which the thiol oxidation was induced should be provided. For example, include the concentrations of DPDSe and ethanol used, incubation times, and buffer conditions. **Comments20:** Author(s) should give Control conditions: It would strengthen this section to mention what control experiments were performed. Were there untreated samples, DPDSe-only samples, and ethanol-only samples for comparison? If these controls were included, it is important to mention them to ensure the reliability of the results. **Experimental Design (In Vivo, Section 2.4)** Comments: Author(s) should Clarify ethanol dose: In Group 4, there seems to be a discrepancy in the ethanol dosing. mention "10 ml/kg of 28% ethanol solution" in this group, but Group 3 was treated with "8 g/kg ethanol." To avoid confusion, provide ethanol doses consistently in terms of either volume or mass, and ensure that the ethanol	

	concentration is clear (e.g., percentage, g/kg). Comments: Author(s) mention that the study was terminated 6 hours after ethanol treatment, but more detail on the experimental timeline would help. For example, at what time points were samples collected for analysis (e.g., right after ethanol administration, 6 hours post-treatment)? Comments: It would be beneficial to explain why a sample size of six rats per group (n=6) was chosen. Were there any power calculations or previous studies that supported this choice? **Lipid Peroxidation Assay (Section 2.5)** Method description: Although TBARS is a common method to measure lipid peroxidation, Author(s) should briefly describe the steps involved in the assay to give a clearer idea of how the MDA-TBA complex formation is quantified. Include details such as incubation time, temperature, and any key reagents or buffers used. Positive control: Indicate whether a positive control (e.g., a known inducer of lipid peroxidation) was used in the TBARS assay. This would help validate the sensitivity and accuracy of the assay. **Thiol Oxidation (Section 2.6)** Comments: Thiol quantification: The method for determining thiol oxidation is briefly described, but the specific techniques or reagents used for quantifying the free SH-groups could be elaborated. Was Ellman's reagent used, or another thiol-specific assay? Mention the specific procedure for "disappearance of SH-groups." **Enzyme Activity Assays (Section 2.7)** Comments: Reaction conditions: The description of the enzyme assays lacks some critical details. Specify the buffer composition, pH, incubation time, and temperature for the assays. Were the reactions performed under standard physiological conditions (e.g., 37°C, pH 7.4)? Comments: Controls for non-enzymatic hydrolysis: Author(s) mention that control experiments were carried out to correct for non-enzymatic hydrolysis of nucleotides. It would be beneficial to describe how these control experiments were designed. Were enzyme inhibitors used, or were heat-inactivated samples run in parallel? Comments: Enzyme activity units: Author(s) report enzyme activities in nmol Pi released/min/mg of protein, but the method used to quantify protein concentration should be mentioned (e.g., Bradford assay, BCA assay). This ensures consistency in normalization. **Gene Expression Analysis (Section 2.8)** Comments: RNA extraction quality: RNA extraction is described using TRI Reagent, but Author(s) should mention whether the RNA quality and concentration were assessed before cDNA synthesis (e.g., via A260/A280 ratio using a spectrophotometer or using gel electrophoresis). High-quality RNA is critical for accurate qPCR results. Comments: cDNA synthesis steps: Clarify the exact amount of RNA used for cDNA synthesis. The text states "1 mg of RNA," which is likely a typo—cDNA synthesis typically uses ng or µg amounts of RNA. **Also, Author(s) should describe** Comments: Deepening the Mechanistic Insights (1) Lipid Peroxidation Mechanism (ii) MDA and Other Markers Comments: Purine Metabolism and Oxidative Stress (I) Enzyme Mechanism (ii) NTPDase Role in Inflammation Comments: Thiol Oxidation and GSH Restoration (i) Thiol Group Significance (ii) Thiol-Specific ROS Detoxification. Comments: Gene Expression: Nrf2 and NF-kB (i) Nrf2 Regulation in Oxidative Stress (ii) NF-kB Pathway (iii) Cross-talk Between Nrf2 and NF-kB	

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PART 2:

	Reviewer's comment	**Author's comment** *(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)*
Are there ethical issues in this manuscript?	*(If yes, Kindly please write down the ethical issues here in details)*	

Reviewer Details:

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