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Journal Name:	**Asian Journal of Research in Biochemistry**
Manuscript Number:	**Ms_AJRB_124542**
Title of the Manuscript:	**THE HAEMATOTOXICITY AND NEPHROTOXICITY EFFECTS OF DIAZEPAM ADMINISTRATION IN MALE ALBINO RATS**
Type of the Article	**Research Article**

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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.	This manuscript, titled "The Haematotoxicity and Nephrotoxicity Effects of Diazepam Administration in Male Albino Rats," provides a well-documented investigation into the potential toxic effects of diazepam on haematological and biochemical indices. The study's relevance is clear, given the widespread clinical use of diazepam and the rising concerns regarding its non-medical use. Strengths: 1. The experimental design is appropriate, with the use of multiple doses of diazepam and varied administration durations (14 and 28 days), which provide a comprehensive view of the drug's impact on haematological and renal parameters. 2. The authors utilized well-established methods for evaluating both biochemical and antioxidant markers, and the inclusion of a detailed materials and methods section ensures reproducibility.	
Is the title of the article suitable? **(If not please suggest an alternative title)**	Yes, the title of the article is clear and suitable.	
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.	The abstract presents the study well, but it can be enhanced for clarity, flow, and impact. **Revised abstract:** Objectives: This study aimed to assess the haematological and nephrotoxic effects of diazepam at varying doses in male albino rats. Materials and Methods: Rats were administered therapeutic (0.062 mg/kg/day), high (0.33 mg/kg/day), and extremely high (0.661 mg/kg/day) doses of diazepam orally for 14 and 28 days. Blood and kidney samples were collected for haematological and biochemical analysis. Results: Diazepam administration significantly reduced red blood cell count, packed cell volume, and platelet count, while other haematological indices were not notably affected. Plasma creatinine and urea levels increased, and total protein decreased across all doses. The treatment also elevated renal thiobarbituric acid reactive substances (TBARS) levels, while reducing antioxidant enzyme activity (SOD, CAT, GSH). Conclusions: Diazepam poses significant risks of oxidative stress, haematotoxicity, and nephrotoxicity. While effective in managing anxiety, caution is necessary due to its potential harmful effects on blood and renal function.	
Are subsections and structure of the manuscript appropriate?	Yes, they are 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.	This manuscript is scientifically robust as it presents a well-designed experimental study assessing the haematotoxic and nephrotoxic effects of diazepam in a controlled animal model. The authors use appropriate dosing regimens that reflect therapeutic, high, and extremely high exposures, which allows for a comprehensive understanding of the dose-response relationship. The methods, including haematological and biochemical analyses, are standard and widely accepted in toxicological studies, ensuring the technical soundness of the results. Additionally, the use of validated oxidative stress markers (e.g., TBARS, antioxidant enzymes) strengthens the reliability of the findings, providing clear evidence of diazepam-induced toxicity.	
Are the references sufficient and recent? If you have suggestions of additional references, please mention them in the review form. **-**	Yes, the references are sufficient and recent**.**	

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Minor REVISION comments **Is the language/English quality of the article suitable for scholarly communications?**	**Areas for Improvement**: • **Introduction**: The introduction provides a broad overview of anxiety disorders and diazepam's role in treatment. However, it would benefit from a more focused discussion on the rationale for exploring haematotoxicity and nephrotoxicity specifically. The current introduction touches on general diazepam toxicity but does not justify the particular focus on blood and kidney markers in this study. A clearer explanation of the significance of diazepam's effects on haematological and renal systems, perhaps with reference to existing literature, would be beneficial. • **Experimental Design**: The methodology used in this study is sound; however, the justification for the specific dose ranges chosen for diazepam administration is not discussed. Are these doses reflective of clinical relevance or aligned with overdose scenarios? Including citations for the dose selection would improve clarity. It would be useful to mention the reasoning behind the choice of male rats exclusively, as the study does not address potential sex-based differences in response to diazepam administration. • **Results**: The results are generally well-presented, but the authors could improve the narrative by better connecting the observed biochemical changes to the potential mechanisms behind diazepam's toxicity. The manuscript mentions oxidative stress as a contributing factor but does not delve into how these changes might interact with kidney or blood functions on a physiological level. A deeper discussion of the relationship between decreased antioxidant enzyme levels (SOD, CAT, GSH) and increased lipid peroxidation (TBARS) would enhance the reader's understanding of how oxidative stress mediates diazepam toxicity. • **Discussion**: The discussion section lacks a robust comparison with prior studies. How do the findings of this study align with or differ from previous research on diazepam's haematotoxicity or nephrotoxicity? A comparison with other drugs in the benzodiazepine class would also be insightful. The study confirms diazepam-induced oxidative stress and toxicity, clarifying how this expands upon the current knowledge base would improve the manuscript's impact. • **Formatting and Clarity**: There are a few grammatical errors and awkward sentence structures, particularly in the introduction (e.g., "the risk it possesses"). Proofreading the manuscript to improve readability would enhance its overall quality. Ensure that figure legends are self-explanatory and provide sufficient detail without the need to refer extensively to the text. • **Conclusion**: The study addresses an important area of research, but it would benefit from a clearer focus on the specific rationale for investigating haematological and renal toxicity in the context of diazepam administration. Additionally, expanding the discussion to connect the biochemical findings with potential physiological mechanisms and previous literature will strengthen the manuscript. Further justification for dose selection and minor revisions in statistical reporting will also enhance its rigor. Once these revisions are addressed, the manuscript will make a valuable contribution to the understanding of diazepam's potential toxic effects.	
Optional/General comments		

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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)*	

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