The ARRIVE guidelines 2.0: author checklist

| Item                                      | Recommendation                                                                 | Section/line number, or reason for not reporting |
|-------------------------------------------|-------------------------------------------------------------------------------|-------------------------------------------------|
| Study design                             | For each experiment, provide brief details of study design including:          | Line 122-125                                    |
|                                           | a. The groups being compared, including control groups. If no control group has | Line 123                                        |
|                                           |   been used, the rationale should be stated.                                  |                                                 |
|                                           | b. The experimental unit (e.g. a single animal, litter, or cage of animals). |                                                 |
| Sample size                              | a. Specify the exact number of experimental units allocated to each group, and | Line 123-124                                   |
|                                           |   the total number in each experiment. Also indicate the total number of animals | Estimation of sample size based on the law of   |
|                                           |   used.                                                                       |   diminishing returns.                          |
|                                           | b. Explain how the sample size was decided. Provide details of any a priori    | Line 123-124.                                  |
|                                           |   sample size calculation, if done.                                          |                                                 |
| Inclusion and exclusion criteria          | a. Describe any criteria used for including and excluding animals (or          | In the process, we included 6-8 weeks male rats | Figure 2,3,4                                   |
|                                           |   experimental units) during the experiment, and data points during the       | and did not set criteria for excluding. Line 108-110. |
|                                           |   analysis. Specify if these criteria were established a priori. If no criteria | no exclusions                                   |
|                                           |   were set, state this explicitly.                                            |                                                 |
|                                           | b. For each experimental group, report any animals, experimental units or data |                                                 |
|                                           |   points not included in the analysis and explain why. If there were no        |                                                 |
|                                           |   exclusions, state so.                                                      |                                                 |
|                                           | c. For each analysis, report the exact value of \( n \) in each experimental   |                                                 |
| Randomisation                            | a. State whether randomisation was used to allocate experimental units to      | Line 125                                        |
|                                           |   control and treatment groups. If done, provide the method used to generate  | Line 123-125.                                  |
|                                           |   the randomisation sequence.                                                |                                                 |
|                                           | b. Describe the strategy used to minimise potential confounders such as the   |                                                 |
|                                           |   order of treatments and measurements, or animal/cage location. If confounders |                                                 |
|                                           |   were not controlled, state this explicitly.                                 |                                                 |
| Blinding                                  | Describe who was aware of the group allocation at the different stages of the  | No blinding was set this study.                 |
|                                           |   experiment (during the allocation, the conduct of the experiment, the        |                                                 |
|                                           |   outcome assessment, and the data analysis).                                |                                                 |
| Outcome measures                         | a. Clearly define all outcome measures assessed (e.g. cell death, molecular    | Section Result.                                 |
|                                           |   markers, or behavioural changes).                                           |                                                 |
|                                           | b. For hypothesis-testing studies, specify the primary outcome measure, i.e.  | Section Method.                                 |
|                                           |   the outcome measure that was used to determine the sample size.             |                                                 |
| Statistical methods                      | a. Provide details of the statistical methods used for each analysis,          | Line 252-258.                                  |
|                                           |   including software used.                                                   |                                                 |
|                                           | b. Describe any methods used to assess whether the data met the assumptions   | Line 252-258.                                  |
|                                           |   of the statistical approach, and what was done if the assumptions were not   |                                                 |
| Experimental animals                     | a. Provide species-appropriate details of the animals used, including species, | Line 122-123.                                  |
|                                           |   strain and substrain, sex, age or developmental stage, and, if relevant,     |                                                 |
|                                           |   weight.                                                                     |                                                 |
|                                           | b. Provide further relevant information on the provenance of animals, health/   | Line 122.                                      |
|                                           |   immune status, genetic modification status, genotype, and any previous       |                                                 |
|                                           |   procedures.                                                                 |                                                 |
| Experimental procedures                  | For each experimental group, including controls, describe the procedures in    | Line 122-125.                                  |
|                                           |   enough detail to allow others to replicate them, including:                 | Fig S1, line 665-667.                          |
|                                           |   a. What was done, how it was done and what was used.                       | 125-126.                                       |
|                                           |   b. When and how often.                                                     | As following Jones B’s study.                   |
|                                           |   c. Where (including detail of any acclimatisation periods).                 |                                                 |
|                                           |   d. Why (provide rationale for procedures).                                 |                                                 |
| Results                                  | For each experiment conducted, including independent replications, report:     | Line 255                                        |
|                                           | a. Summary/descriptive statistics for each experimental group, with a measure | This study, we displayed the data as means ±   |
|                                           |   of variability where applicable (e.g. mean and SD, or median and range).    |   SEM.                                          |
# The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

| Item                      | Recommendation                                                                 | Section/line number, or reason for not reporting |
|---------------------------|-------------------------------------------------------------------------------|-------------------------------------------------|
| **Abstract**              | 11 Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions. | Section Abstract, Line 20-50.                   |
| **Background**            | 12 a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach.  
                           | b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology. | Section Introduction, line 63-100.            |
| **Objectives**            | 13 Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested. | Line 96-100.                                   |
| **Ethical statement**     | 14 Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification. | Line 122-123.                                  |
| **Housing and husbandry** | 15 Provide details of housing and husbandry conditions, including any environmental enrichment. | Line 125-126.                                  |
| **Animal care and monitoring** | 16 a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress.  
                           | b. Report any expected or unexpected adverse events.  
                           | c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this. | Line 138-140.                      |
| **Interpretation/ scientific implications** | 17 a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature.  
                           | b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results. | Section Discussion. Line 423-432. |
| **Generalisability/ translation** | 18 Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate). | Line 416-422.                                  |
| **Protocol registration** | 19 Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered. | Line 131-133.                                  |
| **Data access**           | 20 Provide a statement describing if and where study data are available.       | Section Result                                  |
| **Declaration of interests** | 21 a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated.  
                           | b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study. | Line 444-448.                                  |

Article information: https://dx.doi.org/10.21037/atm-22-542