# The ARRIVE guidelines 2.0: author checklist

## The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

| Item | | Recommendation | Section/line number, or reason for not reporting |
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| **Study design** | 1 | For each experiment, provide brief details of study design including:<br>a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated.<br>b. The experimental unit (e.g. a single animal, litter, or cage of animals). | a. Line: 225 Left (negative and positive control group)<br>b. Line: 223 Left (The experimental unit was a single animal. |
| **Sample size** | 2 | a. Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used.<br>b. Explain how the sample size was decided. Provide details of any *a priori* sample size calculation, if done. | a. Line 223 Left (Each group consisted of 6 rats, with a total of 24 rats)<br>b. Line 223 Left: The sample size was determined using Federer's formula. |
| **Inclusion and exclusion criteria** | 3 | a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established *a priori*. If no criteria were set, state this explicitly.<br>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.<br>c. For each analysis, report the exact value of *n* in each experimental group. | a. Line 137 Right (Male Wistar rats aged 2–3 months and weighing 200–250 g were used and acclimatized prior to the experiment. Only rats with total cholesterol levels >100 mg/dL after induction were included in the study.)<br>b. Line 199 Left: No animals or data were excluded from the analysis.<br>c. Line 223 Left (The exact value of n was 6 rats per group for the antihyperlipidemic analysis, with a total of 24 rats across four groups) |
| **Randomisation** | 4 | a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence.<br>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. | a. Line 225 Left: The rats were randomly assigned using a computer based random order generator into four groups<br>b. Line 232 Left: Potential confounders were minimized by standardizing the timing of treatment administration and measurements, maintaining identical housing and environmental conditions, and applying consistent handling procedures throughout the study. |

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| **Blinding** | 5 | Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis). | Line 203 Right: Blinding was not applied during group allocation or treatment administration; however, biochemical outcome measurements and data analysis were performed by an investigator blinded to the group allocation to minimize bias. |
| **Outcome measures** | 6 | a. Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes).<br>b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size. | a. Line 135 Right: The outcome measures assessed included total cholesterol and triglyceride levels.<br>b. Line 135 Right: The outcome measures assessed included total cholesterol and triglyceride levels. |
| **Statistical methods** | 7 | a. Provide details of the statistical methods used for each analysis, including software used.<br>b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met. | a. Line 210 Right<br>b. Line 210 Right<br><br>The total cholesterol and triglyceride data were statistically analyzed using a two-way ANOVA (SPSS version 30) followed by Duncan's multiple range test to evaluate the effects of treatment group and administration duration, as well as their interaction, on lipid levels. Normality and homogeneity of variance were assessed using the Shapiro–Wilk and Levene's tests, respectively. |
| **Experimental animals** | 8 | a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight.<br>b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures. | a: Line 137 Right<br>b: Line 137 Right<br>Healthy male Wistar rats aged 2–3 months and weighing 200–250 g were obtained from the Laboratory Animal Centre at the Faculty of Pharmacy, Universitas Andalas |
| **Experimental procedures** | 9 | For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including:<br>a. What was done, how it was done and what was used.<br>b. When and how often.<br>c. Where (including detail of any acclimatisation periods).<br>d. Why (provide rationale for procedures). | a. Line 246: Hyperlipidemia induction<br>Line 246: Administration of the sample test<br>Line 246: Sample analysis<br>b. Line 246: Duration of hyperlipidemia induction<br>Line 246: Duration of sample test administration<br>Line 251: Time of sample collection<br>c. Line 205: Before treatment, rats were acclimatized for 7 days at the Laboratory Animal Center under standard laboratory conditions with free access to food and water to allow adaptation to the environment. Experimental procedures were conducted at the Laboratory of Anatomy and Pharmacology and the Laboratory Animal Center.<br>d. Line 199: Rationale for the use of male rats |

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| | | | Line 365: Rationale for measuring total cholesterol and triglyceride levels |
| **Results** | 10 | For each experiment conducted, including independent replications, report:<br>a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range).<br>b. If applicable, the effect size with a confidence interval. | a. Line 240: Descriptive statistics were reported as mean ± SD for each experimental group.<br>b. Effect size and confidence intervals were not calculated. |

# 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. | line: 11, 12, 19 |
| **Background** | 12 | a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach.<br>b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology. | a. line: 34 left, 44 left, 34 right antihyperlipidemic potential of piperine and the use of nano-cocrystal technology to improve its bioavailability<br>b. line 109 left |
| **Objectives** | 13 | Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested. | Line 97 Left |
| **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 585 |
| **Housing and husbandry** | 15 | Provide details of housing and husbandry conditions, including any environmental enrichment. | Line 205 |
| **Animal care and monitoring** | 16 | a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress.<br>b. Report any expected or unexpected adverse events.<br>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 205 |
| **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.<br>b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results. | a. Line 373 right<br>b. Line 485 right |
| **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). | The findings of this study suggest that piperine–succinic acid nano-cocrystals may have potential for use under conditions associated with dyslipidemia. Since the materials used in the formulation are considered safe for human use, these results may provide preliminary information for determining appropriate cocrystal doses in future human studies. However, further investigations are still needed to confirm their efficacy and safety in humans. |
| **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. | A study protocol including the research objectives, hypothesis, experimental design, and analysis plan was prepared prior to the study. The study hypothesized that the piperine–succinic acid cocrystal formulation could improve piperine |

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| | | | solubility and enhance its antihyperlipidemic activity; however, the protocol was not publicly registered. |
| **Data access** | 20 | Provide a statement describing if and where study data are available. | Line 580 |
| **Declaration of interests** | 21 | a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated.<br>b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study. | a. Line 590 left<br>b. Line 519 right |