National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

The ARRIVE Guidelines

Animal Research: Reporting of *In Vivo* Experiments

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The ARRIVE (Animal Research: Reporting of *In Vivo* Experiments) guidelines were developed as part of an NC3Rs initiative to improve the design, analysis and reporting of research using animals – maximising information published and minimising unnecessary studies. The guidelines were published in the online journal *PLOS Biology* in June 2010 and are currently endorsed by scientific journals, major funding bodies and learned societies.

The guidelines are intended to:

- Improve reporting of research using animals.
- Guide authors as to the essential information to include in a manuscript, and not be absolutely prescriptive.
- Be flexible to accommodate reporting a wide range of research areas and experimental protocols.
- Promote reproducible, transparent, accurate, comprehensive, concise, logically ordered, well written manuscripts.
- Improve the communication of the research findings to the broader scientific community.

The guidelines are NOT intended to:

- Promote uniformity, stifle creativity, or encourage authors to adhere rigidly to all items in the checklist. Some of the items may not apply to all studies, and some items can be presented as tables/figure legends or flow diagrams (e.g. the numbers of animals treated, assessed and analysed).
- Be a guide for study design and conduct. However, some items on the checklist, such as randomisation, blinding and using comparator groups, may be useful when planning experiments as their use will reduce the risk of bias and increase the robustness of the research.

Who are the guidelines aimed at?

- Novice and experienced authors
- Journal editors
- Peer reviewers
- Funding bodies

What kind of research areas do the guidelines apply to?

- The guidelines will be most appropriate for comparative studies, where two or more groups of experimental animals are being compared; often one or more of the groups may be considered as a control. They apply also to studies comparing different drug doses, or, for example, where a single animal is used as its own control (within-subject experiment).
- Most of the recommendations also apply to studies that do not have a control group.
- The guidelines are suitable for any area of bioscience research where animals are used.

How might these guidelines be used?

The guidelines provide a checklist for those preparing or reviewing a manuscript intended for publication.

References

1. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG (2010) Improving Bioscience Research Reporting: The ARRIVE Guidelines for Reporting Animal Research. *PLOS Biol* 8(6): e1000412. doi:10.1371/journal.pbio.1000412
2. Schulz KF, Altman DG, Moher D, the CONSORT Group (2010) CONSORT 2010 Statement: updated guidelines for reporting parallel group randomised trials. *BMJ* 340:c332.

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Further Information

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	ITEM	RECOMMENDATION
Title	1	Provide as accurate and concise a description of the content of the article as possible. Pls see pg 1, lines 1-2
Abstract	2	Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study. Pls see pg 3, lines 1-23; pg 4, lines 1-12
INTRODUCTION		
Background	3	a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale. b. Explain how and w… the scientific object… human biology. In the present study, MCAO-induced embolic stroke in rat model is reported to mimics human strokes more closely than other models of cerebral ischemia (Overgaard, Cerebrovasc Brain Metabol Rev 1994; 6:257-286).
Objectives	4	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested. Pls see pg 5,lines 22-23
METHODS		
Ethical statement	5	Indicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research. Pls see pg 6, line 23; pg 7, lines 1-3
Study design	6	For each experiment, give brief details of the study design including: a. The number of experimental and control groups. Pls see pg 7, lines 11-16 b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when). Pls see pg 7, lines 19-21 c. The experimental unit (e.g. a single animal, group or cage of animals). Pls see pg 7, lines 9-12 A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.
Experimental procedures	7	For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). Pls see pg 7, lines 4-16 b. When (e.g. time of day). Pls see pg 7, lines 4-16 c. Where (e.g. home cage, laboratory, water maze). Pls see pg 7, lines 3-7 d. Why (e.g. rationale… drug dose used). On the day of surgery, animals were anesthetized with a mixture of 75% air and 25% O_2 gases containing 3% isoflurane. We had mentioned this information in version 1 (V1) of the manuscript, however the editor or editing department asked us to remove. Thus we removed in the version 6 and mentioned as at 24 h after reperfusion, the rats were anesthetized in pg 8, line 7.
Experimental animals	8	a. Provide details of … developmental stage… (e.g. mean or median weight plus weight range). Pls see pg 6, line 23. b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc. Animals were purchased from BioLASCO Taiwan Co., Ltd. for this study.
Housing and husbandry	9	Provide details of: a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish). Pls see pg 6, lines 4-11. Polypropylene cages and hardwood chips bedding materials were used for housing the animals 5 in each cage. b. Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment). Pls see pg 7, lines 6-7. c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment.
Sample size	10	a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group. Pls see pg 7, lines 4-16. b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used. The rats were randomly separated into five groups of five rats each as shown in page 7, lines 11-12. c. Indicate the number of independent replications of each experiment, if relevant.
Allocating animals to experimental groups	11	a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done. The rats were randomly separated into five groups of five rats each as shown in page 7, lines 11-12. b. Describe the order in which the animals in the different experimental groups were treated and assessed. Pls see pg 7, lines 11-16.
Experimental outcomes	12	Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes). Pls see pg 7, lines 19-23; pg 8, lines 1-4
Statistical methods	13	a. Provide details of the statistical methods used for each analysis. b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). c. Describe any methods used to assess whether the data met the assumptions of the statistical approach. Pls see pg 10, lines 7-11.
RESULTS		
Baseline data	14	For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naïve) prior to treatment or testing (this information can often b… The mortality of rats was not observed, and the body weight not changed markedly from the initial weight. No infection or inflammation were found prior to , during or after treatment.
Numbers analysed	15	a. Report the number of animals in each group inc… absolute numbers (e.g. 10/20, not 50%[2]). b. If any animals or data were not included in the analysis, explain why.
		Pls see pg 10, lines 14-23; pg 11, lines 1-11; pg 12, lines 1-24; pg 13, lines 1-2 Report the results for each analysis carried o… (e.g. standard error or confidence interval). The rats were randomly separated into five groups of five rats each as shown in page 7, lines 11-12.
Adverse events	17	a. Give details of all important adverse events in each experimental group. No found any adverse events during the experimental periods b. Describe any modifications to the experimental protocols made to reduce adverse events.
DISCUSSION		
	18	Pls see pg 13, lines 5-23; pg 14, lines 1-22; pg 15, lines 1-8 a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. In this study, there is no limitation using animal model, as we used normal Wistar rats. b. Comment on the study limitations including any potential sources of bias, any limitations of the animal model, and the imprecision associated with the results[2]. No implication c. Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research.
…heralisability/ …nslation	19	Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology.
Funding	20	List all funding sources (including grant number) and the role of the funder(s) in the study. Pls see pg 16, lines 10-12

The results of the present study is important for understanding the neuroprotective mechanism of TRCQT and supporting its clinical therapeutic value as it is reported that MCAO-induced embolic stroke in rat model is highly mimics human strokes and more closely than other models of cerebral ischemia (Overgaard, Cerebrovasc Brain Metabol Rev 1994; 6:257-286).