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| ITEM RECOMMENDATION | | | |
| Title | 1 | Provide as accurate and concise a description of the content of the article as possible | Title |
| 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. | Abstract |
| 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 why the animal species and model being used can address  the scientific objectives and, where appropriate, the study’s relevance to  human biology. | paragraph 1-3 |
| Objectives | 4 | Clearly describe the primary and any secondary objectives of the study, or  specific hypotheses being tested. | paragraph 3 line6-7 |
| 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. | Ethics approval and consent to participate |
| Study design | 6 | For each experiment, give brief details of the study design including:  a. The number of experimental and control groups.  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).  c. The experimental unit (e.g. a single animal, group or cage of animals).  A time-line diagram or flow chart can be useful to illustrate how complex study  designs were carried out. | Materials and Methods 2.6 |
| 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).  b. When (e.g. time of day).  c. Where (e.g. home cage, laboratory, water maze).  d. Why (e.g. rationale for choice of specific anaesthetic, route of administration,  drug dose used). | Materials and Methods 2.6 |
| Experimental  animals | 8 | a. Provide details of the animals used, including species, strain, sex,  developmental stage (e.g. mean or median age plus age range) and weight  (e.g. mean or median weight plus weight range).  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 | Materials and Methods 2.6 |
| 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).  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).  c. Welfare-related assessments and interventions that were carried out prior to,  during, or after the experiment. | Materials and Methods 2.6 |
| Sample size | 10 | a. Specify the total number of animals used in each experiment, and the number  of animals in each experimental group.  b. Explain how the number of animals was arrived at. Provide details of any sample  size calculation used.  c. Indicate the number of independent replications of each experiment, if relevant. | Materials and Methods 2.6 |
| Allocating animals  to experimental  groups | 11 | a. Give full details of how animals were allocated to experimental groups, including  randomisation or matching if done.  b. Describe the order in which the animals in the different experimental groups  were treated and assessed. | Materials and Methods 2.6 |
| Experimental  outcomes | 12 | Clearly define the primary and secondary experimental outcomes assessed  (e.g. cell death, molecular markers, behavioural changes). | Materials and Methods 2.6 |
| 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. | Materials and Methods 2.10 |
| 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 be tabulated). | Results 3.3 Body weight gain |
| Numbers analysed | 15 | a. Report the number of animals in each group included in each analysis. Report  absolute numbers (e.g. 10/20, not 50% 2 ).  b. If any animals or data were not included in the analysis, explain why. | Results 3.3, 3.4,3.5 |
| Outcomes and  estimation | 16 | Report the results for each analysis carried out, with a measure of precision  (e.g. standard error or confidence interval). | Results 3.3, 3.4,3.5 |
| Adverse events | 17 | a. Give details of all important adverse events in each experimental group.  b. Describe any modifications to the experimental protocols made to reduce  adverse events. | N/A |
| DISCUSSION | | | |
| Interpretation/  scientific implications | 18 | 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 any potential sources of bias, any  limitations of the animal model, and the imprecision associated with the results 2 .  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. | Discussion paragraph 1-2 |
| Generalisability/  translation | 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 | Discussion paragraph 3 |
| Funding | 20 | List all funding sources (including grant number) and the role of the funder(s)  in the study. | Funding |
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