Reviewer #1:

1. First of all, it is necessary to improve figure quality and increase dimensions of the figures which are hardly readable.

   We are extremely grateful for your valuable opinions and suggestions. Your suggestion has been very helpful in enhancing the quality of our manuscript. In response to your initial concerns about the figure quality, we have immediately taken steps to increase the figure dimensions and have adjusted the size to ensure readability and clarity. The updated data has been added into the revised manuscript. Thank you once again for your guidance.

2. In all microscope images there is no indication about the scale bar.

   Thank you for your valuable suggestion. We understand your concern regarding the lack of scale bars in the micrographs, which is crucial for the accurate interpretation of the images. We acknowledge that the scale bars in the original figures were indeed too small and not conspicuous. Since the scale is embedded within the image, the properties of the scale cannot be altered. Following your suggestion, we have added locally magnified images below the original images to ensure that they are clearly discernible in all micrographs. This adjustment has been made without altering the original data or image quality. We have paid close attention to maintaining the integrity and clarity of the images to facilitate better understanding for the readers. The revised data are now included in the revised manuscript. As shown in the figure below. But, during the process of capturing images, we inadvertently used different scale formats at the same magnification, which did not affect the actual magnification of the images. We have come to deeply appreciate the importance of consistency in the presentation of scales used at the same magnification. Therefore, we will pay attention to this detail in future image acquisitions to ensure uniformity and clarity. We would like to express our gratitude once again for your constructive suggestion on our research.
3. Both in cell and animal studies, there is no quantification of Oil Red O or hematoxylin eosin.

Thank you for your valuable suggestion. The quantitative analysis of Oil Red O and hematoxylin and eosin (H&E) staining have been added in our revised manuscript (Figure 1D, 3I-J). The incorporation of these quantitative data strengthens the validity of our findings, and we would like to express our gratitude once again for your suggestion. As shown in the figure below.
Data are presented as mean ± SE. *P < 0.05 vs NC group, †P < 0.01 vs NC group, ‡P < 0.05 vs MOD group, §P < 0.01 vs MOD group

4. there is no indication in methods about what is liver/fat index which are shown in fig.3.

Thank you for your insightful suggestion. The calculation of the fat index and liver index have been added in the "Methods" section of the revised manuscript. The specific details are as follows: The fat index in mice was calculated by determining the ratio of the fat tissue weight to the body weight. The specific formula for the fat index was: Fat Index = weight of fat tissue (mg)/ body weight of mice (g) The liver index was ascertained by dividing the liver weight by the body weight of the mice. The formula for calculating the liver index was: Liver Index = weight of liver tissue (mg)/ body weight of mice (g).

5. Also, a quantification of TUNEL assay in fig 8 is missing.

Thank you for your valuable suggestion. The quantitative analysis of TUNEL assay has been added in our revised manuscript. The incorporation of these quantitative data strengthens the validity of our findings (Figure 9D), and we would like to express our gratitude once again for your suggestion. As shown in the figure below.
Data are presented as mean ± SE. aP < 0.05 vs NC group, bP < 0.01 vs NC group, cP < 0.05 vs MOD group, dP < 0.01 vs MOD group

6. It could be necessary a quantification of phosphorylated forms of AMPKa and ACC; to evaluate if there is some change in their level of activation and not only expression.

Thank you for your valuable suggestion. We have now included a detailed quantitative analysis of phosphorylated forms of AMPKa and ACC1 in our revised manuscript (Figure 6A). The incorporation of these quantitative data strengthens the validity of our findings, and we would like to express our gratitude once again for your suggestion. As shown in the figure below.

A

Data are presented as mean ± SE. aP < 0.05 vs NC group, bP < 0.01 vs NC group, cP < 0.05 vs MOD group, dP < 0.01 vs MOD group

7. I suggest to add a paragraph of limitations: there is no evaluation of effect of sex in these studies.

We thank you for your comments on the necessity of assessing the impact of the drug on gender differences. We unanimously agree that understanding the potential gender-specific effects is vital for a comprehensive evaluation of the drug’s efficacy and safety. We acknowledge that our study did not
initially consider gender as a variable, and we recognize the significance of this oversight. Therefore, in our future research work, we will include the impact of the drug on gender as a direction of investigation. This will enable us to gain a more nuanced understanding of the drug's effects across different genders and contribute to adopting a more equitable approach to medical treatment. Additionally, the lack of gender assessment in this study is described in the conclusion of the revised manuscript. The specific details are as follows:

The present study had some limitations. First, we did not assess the impact of FLHZF on gender, safety, and side effects. There may be gender differences in the effects of FLHZF, as well as potential safety and side effect issues. These aspects are worth in-depth study in future research, which will contribute to a more comprehensive understanding of the clinical application of FLHZF.

8. Justification about the doses used in cell and mice may be needed: are they based on previous studies?

Thank you for your valuable suggestion.

In cell experiments, the administration dose of Fanlian Huazhuo Formula was determined based on two aspects. The first was the effect of Fanlian Huazhuo Formula on the content of Triglycerides (TG, Triglycerides) in HepG2 cells. The results are shown in the figure below: Fanlian Huazhuo Formula did not cause a significant increase the content of TG in HepG2 cells without free fatty acids, indicating that these concentrations may reduce TG accumulation in HepG2 cells with free fatty acids.

![Graph showing TG levels with different doses of FLHZF](image)

The second is that at these concentrations of FLHZF, cell viability compromised by free fatty acid induction was effectively enhanced, and TG levels were significantly reduced. As shown in the following results (Figure 1A-C):
Data are presented as mean ± SE. \(^a\)P < 0.05 vs NC group, \(^b\)P < 0.01 vs NC group, \(^c\)P < 0.05 vs MOD group, \(^d\)P < 0.01 vs MOD group

In animal experiments, the dosage administered to mice is calculated based on the clinically equivalent dose, using the following method:

The low dose of FLHZF administered per gram of mouse per day is calculated as \(\frac{175 \text{ g/d}}{70 \text{ kg}} \times 9.1 = 22.75 \text{ g/(kg d)}\). and the high dose was calculated as \(\frac{175 \text{ g/d}}{70 \text{ kg}} \times 9.1 \times 2 = 45.5 \text{ g/(kg d)}\); (175 g is the daily dosage for an adult, 70 kg is the average weight of an adult, and 9.1 is the coefficient). And the above calculation method is also added to the method of animal study in the revised manuscript, so that readers can be clearer about the dosage of Fanlian Huazhuo Formula in mice.

Thank you for your valuable suggestions. After carefully reviewing your proposed references, we were impressed by the depth and level of research in each one. Based on editorial suggestions and taking into account the research direction of our manuscript, we selected two references that were most relevant to the revised manuscript. The cited references are numbered 56 and 62 in the revised manuscript. These selections have been integrated into our manuscript to improve its depth and relevance.

We are deeply interested in the articles you have written about non-alcoholic fatty liver disease (NAFLD) and will continue to follow your research outcomes. We hope that through your professional insights and in-depth analysis, we can gain a more comprehensive understanding of the causes, development process, and possible prevention and treatment strategies for this disease. We believe that as our knowledge in this field continues to accumulate, it will help us make more active contributions to addressing this increasingly serious health issue in our future research and practice.

Thank you again for your valuable suggestions.

Reviewer #2

1. Consider providing a rationale for the selection of specific dosages of FLHZF and the duration of treatment. This would enhance the transparency of the study's methodology and provide valuable insights into the optimal therapeutic regimen for FLHZF in NAFLD management.

Thank you for your constructive suggestions. We have carefully considered your suggestions and now provide the following detailed response:

Fanlian Huazhuo Formula has been in clinical use for many years. We determined the dosage based on the body surface area conversion formula between humans and mice, which is a standard method to ensure dosages are appropriately scaled for preclinical studies.
The low dose of FLHZF administered per gram of mouse per day is calculated as = (175 g/d)/70 kg × 9.1 = 22.75 g/(kg d). and the high dose was calculated as = (175/d)/70 kg × 9.1 × 2 = 45.5 g/(kg d); (175 g is the daily dosage for an adult, 70 kg is the average weight of an adult, and 9.1 is the coefficient). And the above calculation method is also added to the method of animal study in the revised manuscript, so that readers can be clearer about the dosage of Fanlian Huazhuo Formula in mice.

Treatment duration of Fanlian Huazhuo Formula in vivo experiments: Combining the clinical treatment duration of Fanlian Huazhuo Formula (12 wk) and the serum marker results of non-alcoholic fatty liver disease mice at the 10 wk, we determined our treatment period. We observed that after 10 wk of treatment, there was a significant improvement in the serum markers of the mice. Therefore, we chose 10 weeks as the treatment duration for mice with NAFLD.

The concentrations of Fanlian Huazhuo Formula in vitro experiments: We selected drug concentrations of 25, 50, 100, and 200 μg/mL. At these concentrations, the cell viability induced by free fatty acids was effectively enhanced, and the level of triglyceride was significantly reduced (Figure 1A-C).
Data are presented as mean ± SE. \(^aP < 0.05\) vs NC group, \(^bP < 0.01\) vs NC group, \(^cP < 0.05\) vs MOD group, \(^dP < 0.01\) vs MOD group.

Treatment duration of Fanlian Huazhuo Formula in vitro experiments: Through the evaluation of the positive area ratio of Oil Red O staining under various treatment durations and drug concentrations. We found that the optimal therapeutic effect was appeared when the treatment concentrations of the Fanlian Huazhuo Formula were 25, 50, 100, and 200 μg/mL, and the treatment duration was 24 h. The results are shown in the figure below. Based on these findings, we selected the aforementioned concentrations and treatment times for our cellular experiments to ensure that our in vitro treatment conditions are effective.
2. It is recommended to incorporate blinding procedures into future studies to minimize potential bias during data collection and analysis. Implementing blinding methods, such as blinded assessment of outcomes or data analysis by blinded researchers, would enhance the methodological rigor of the study.

We are very grateful for your valuable suggestion. We fully agree with your suggestion, and as such, we have discussed the necessity of including a blinding procedure in the final paragraph of the discussion section of revised manuscript. We will adopt this recommendation in our future research and ensure that the implementation of the blinding procedure is thoroughly considered in the research design and execution process. This will help us to provide more accurate and reliable research outcomes. Thank you again for your advice. As shown below future research will add blinding procedures to ensure that the results are objective and not influenced by subjective factors.

Thanks again for your advice.

3. Expand the discussion to include a thorough assessment of FLHZF’s safety profile and potential side effects. Addressing safety considerations and discussing any observed adverse events associated
with FLHZF treatment would provide a more comprehensive evaluation of its therapeutic potential and support informed decision-making in clinical practice.

We are very grateful for your opinions and suggestions. We also pay great attention to the safety of the medication. Therefore, we will add into this issue in subsequent research. This will help to provide a more comprehensive assessment of the safety, side effects, and adverse events of FLHZF, and will contribute to better therapeutic effects of FLHZF in clinical applications. The description of this part has been supplemented in the last paragraph of the discussion in the revised manuscript, which describes that future research will focus on the safety issues related to FLHZF. As shown below the present study had some limitations. First, we did not assess the impact of FLHZF on gender, safety, and side effects. There may be gender differences in the effects of FLHZF, as well as potential safety and side effect issues. These aspects are worth in-depth study in future research, which will contribute to a more comprehensive understanding of the clinical application of FLHZF.

Thanks again for your advice.

4. In the conclusion, consider outlining specific directions for future research, such as clinical trials or mechanistic studies, to further investigate FLHZF's efficacy and underlying mechanisms of action. Providing clear guidance on future research directions would help advance the understanding of FLHZF's therapeutic benefits and its potential applications in clinical settings.

Thank you very much for your valuable advice. We have carefully considered your comments and have added a description of future research directions to the conclusion of the revised manuscript. Through these additional future research directions, we expect to be able to provide new perspectives and strategies for the research and treatment of metabolic diseases, while opening up new paths for the modern research and application of traditional medicines such as FLHZF.

The description in the revised manuscript is as follows:

Our study broadens the application of FLHZF and offers an additional treatment option for NAFLD. In the future, metabolomics, will be used to study the specific effects of FLHZF in NAFLD. We aim to analyze the mechanisms by which FLHZF affects metabolic pathways and explore the relationship between these metabolic changes and metabolic diseases such as NAFLD, thereby offering new
perspectives and strategies for their prevention and treatment.

Thanks again for your advice.

5. Strengthen the study's conclusions by including additional control groups or comparisons, such as positive control groups or comparisons with existing treatments for NAFLD. This would provide a more robust context for interpreting FLHZF's therapeutic effects and enhance the study's overall scientific validity.

Thank you for your suggestion. We have added the content of this pharmacodynamic comparison in the conclusion section of the revised manuscript, as shown below: Comparison of the therapeutic effects between the FLHZF-H and fenofibrate groups in the pharmacodynamic experiments showed that, in terms of reducing weight gain, abdominal fat index and genital fat index, as well as serum ALT levels, the FLHZF-H group demonstrated better efficacy. In terms of reducing liver index, serum AST levels, NAS score, and the proportion of Oil Red O staining positive areas, there was no difference in the therapeutic effects between the FLHZF-H and fenofibrate groups. Therefore, FLHZF has superior therapeutic effects in multiple aspects, providing strong evidence for further research into its potential for treating NAFLD.

The statistical analysis results for the two groups are as follows:
Data are presented as mean ± SE. *P < 0.05 vs NC group, **P < 0.01 vs NC group, ***P < 0.05 vs MOD
group, $^dP < 0.01$ vs MOD group, $^eP < 0.05$ vs FNBT group and FLHZF-H group.

Thanks again for your advice.

(1) Science Editor:

1. The impact of gender was not evaluated in these studies.

We thank you for your comments on the necessity of assessing the impact of the drug on gender differences. We unanimously agree that understanding the potential gender-specific effects is vital for a comprehensive evaluation of the drug's efficacy and safety. We acknowledge that our study did not initially consider gender as a variable, and we recognize the significance of this oversight. Therefore, in our future research work, we will include the impact of the drug on gender as a direction of investigation. This will enable us to gain a more nuanced understanding of the drug's effects across different genders and contribute to adopting a more equitable approach to medical treatment. Additionally, the lack of gender assessment in this study is described in the conclusion of the revised manuscript. The specific details are as follows:

The present study had some limitations. First, we did not assess the impact of FLHZF on gender, safety, and side effects. There may be gender differences in the effects of FLHZF, as well as potential safety and side effect issues. These aspects are worth in-depth study in future research, which will contribute to a more comprehensive understanding of the clinical application of FLHZF.

2. In addition, it may be necessary to demonstrate the dosage used in cells and mice: are they based on previous studies?

Thank you for your constructive suggestions. We have carefully considered your suggestions and now provide the following detailed response:

Fanlian Huazhuo Formula has been in clinical use for many years. We determined the dosage based on the body surface area conversion formula between humans and mice, which is a standard method to ensure dosages are appropriately scaled for preclinical studies.

The low dose of FLHZF administered per gram of mouse per day is calculated as $=(175 \text{ g/d})/70 \text{ kg}$
× 9.1 = 22.75 g/(kg d). and the high dose was calculated as = (175/d)/70 kg × 9.1 × 2 = 45.5 g/(kg d); (175 g is the daily dosage for an adult, 70 kg is the average weight of an adult, and 9.1 is the coefficient).

And the above calculation method is also added to the method of animal study in the revised manuscript, so that readers can be clearer about the dosage of Fanlian Huazhuo Formula in mice.

The concentrations of Fanlian Huazhuo Formula in vitro experiments: We selected drug concentrations of 25, 50, 100, and 200 μg/mL. At these concentrations, the cell viability induced by free fatty acids was effectively enhanced, and the level of triglyceride was significantly reduced (Figure 1A-C).

![Cell viability](image1)

![TG](image2)

Data are presented as mean ± SE. \(^a P < 0.05\) vs NC group, \(^b P < 0.01\) vs NC group, \(^c P < 0.05\) vs MOD group, \(^d P < 0.01\) vs MOD group

3. Consider providing reasons for selecting specific doses and treatment durations for FLHZF.
Thank you for your constructive suggestions. We have carefully considered your suggestions and now provide the following detailed response:

Fanlian Huazhuo Formula has been in clinical use for many years. We determined the dosage based on the body surface area conversion formula between humans and mice, which is a standard method to ensure dosages are appropriately scaled for preclinical studies.

The low dose of FLHZF administered per gram of mouse per day is calculated as \( \frac{175 \, \text{g}}{70 \, \text{kg}} \times 9.1 = 22.75 \, \text{g}/(\text{kg} \cdot \text{d}) \), and the high dose was calculated as \( \frac{175}{70 \, \text{kg}} \times 9.1 \times 2 = 45.5 \, \text{g}/(\text{kg} \cdot \text{d}) \); (175 g is the daily dosage for an adult, 70 kg is the average weight of an adult, and 9.1 is the coefficient). And the above calculation method is also added to the method of animal study in the revised manuscript, so that readers can be clearer about the dosage of Fanlian Huazhuo Formula in mice.

Treatment duration of Fanlian Huazhuo Formula in vivo experiments: Combining the clinical treatment duration of Fanlian Huazhuo Formula (12 wk) and the serum marker results of non-alcoholic fatty liver disease mice at the 10 wk, we determined our treatment period. We observed that after 10 wk of treatment, there was a significant improvement in the serum markers of the mice. Therefore, we chose 10 weeks as the treatment duration for mice with NAFLD.

The concentrations of Fanlian Huazhuo Formula in vitro experiments: We selected drug concentrations of 25, 50, 100, and 200 \( \mu \text{g/mL} \). At these concentrations, the cell viability induced by free fatty acids was effectively enhanced, and the level of triglyceride was significantly reduced (Figure 1A-C).
Data are presented as mean ± SE. *P < 0.05 vs NC group, \( \text{a} P < 0.01 \) vs NC group, \( \text{c} P < 0.05 \) vs MOD group, \( \text{d} P < 0.01 \) vs MOD group

Treatment duration of Fanlian Huazhuo Formula \textit{in vitro} experiments: Through the evaluation of the positive area ratio of Oil Red O staining under various treatment durations and drug concentrations. We found that the optimal therapeutic effect was appeared when the treatment concentrations of the Fanlian Huazhuo Formula were 25, 50, 100, and 200 \( \mu \text{g/mL} \), and the treatment duration was 24 h. The results are shown in the figure below. Based on these findings, we selected the aforementioned concentrations and treatment times for our cellular experiments to ensure that our \textit{in vitro} treatment conditions are effective.
4. It is recommended to incorporate blinding procedures into future studies to minimize potential bias during data collection and analysis;

We are very grateful for your valuable suggestion. We fully agree with your suggestion, and as such, we have discussed the necessity of including a blinding procedure in the final paragraph of the discussion section of revised manuscript. We will adopt this recommendation in our future research and ensure that the implementation of the blinding procedure is thoroughly considered in the research design and execution process. This will help us to provide more accurate and reliable research outcomes. Thank you again for your advice. As shown below future research will add blinding procedures to ensure that the results are objective and not influenced by subjective factors.

Thanks again for your advice.

5. References recommendations: The peer reviewer requested the author to cite their own article "Administration of Linoleoylthalamide Reduced Weight Gain", Dyslipidemia, and Inflammation Associated with High-Fat-Diet-Induced Obesity", after verification, its theme is related to the manuscript. The reviewer didn’t request the authors to cite improper references published by him/herself.
Thank you for your valuable suggestions. After carefully reviewing your proposed references, we were impressed by the depth and level of research in each one. Based on editorial suggestions and taking into account the research direction of our manuscript, we selected two references that were most relevant to the revised manuscript. The cited references are numbered 56 and 62 in the revised manuscript. These selections have been integrated into our manuscript to improve its depth and relevance.

Thank you again for your valuable suggestions.

6. The language classification is Grade B and Grade B. Please provide the latest Language certificate after Return the Manuscript to Author for Revision. Please visit the following website for the professional English language editing companies that we recommend: https://www.wjgnet.com/bpg/gerinfo/240.

Thank you for your guidance and the recommended language editing companies. I have reviewed the website you provided. After careful consideration, I have selected a professional English language editing service from the recommended list to ensure that my manuscript meets the publication's standards. Upon completion of the editing process, I will promptly submit the revised manuscript along with the latest language certificate as requested. Thank you for your assistance and support throughout this process.

Thank you again for your valuable suggestions.

(3) Author list. A hyphen should be included between the syllables of Chinese names. For example: Yi-Fan Chang, Jia-Jing Li, Tao Liu, Chong-Qing Wei, Li-Wei Ma, Vladimir N Nikolenko, Wei-Long Chang.

Thank you for your suggestion, which has been incorporated into the manuscript with the following modifications:

Meng-Yuan Niu, Geng-Ting Dong, Yi Li, Qing Luo, Liu Cao, Xi-Min Wang, Qi-Wen Wang, Yi-Ting Wang, Zhe Zhang, Xi-Wen Zhong, Wei-Bo Dai, Le-Yu Li
Thank you again for your valuable suggestions.

(4) Policy of allowing co-first authors and co-corresponding authors who made equal contribution to a manuscript (https://www.wjgnet.com/bpg/GerInfo/310).

Thank you for your suggestion, which has been added into the revised manuscript with the following modifications:

Niu MY and Dong GT were responsible for designing the experiments, analyzing the data, and writing the manuscript. Dai WB and Li LY were responsible for providing the experimental design and financial support.

Thank you again for your valuable suggestions.

(5) Author contributions does not meet the requirements: The ‘Author contributions’ passage describes the specific contribution(s) made by each author. The author’s names will be listed in the following format: full family (sur)name, followed by abbreviated first and middle names. For example, Bryan L Copple should be revised as Copple BL. A full multi-author example is: Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research study; Wang CL, Zou CC, Hong F and Wu XM performed the research.

Thank you for your suggestions, the appropriate revisions have been made in the revised manuscript.

Author contributions: Niu MY and Dong GT were responsible for designing the experiments, analyzing the data, and writing the manuscript. Niu MY and Li Y conducted the experimental research. Luo Q, Cao L, Wang XM, Wang QW, Wang YT, Zhang Z, and Zhong XW provided the research software and experimental methods. Dai WB and Li LY were responsible for providing the experimental design and financial support. All data were generated in-house, and no paper was used. All authors agree to be accountable for all aspects of work ensuring integrity and accuracy.

Thank you again for your valuable suggestions.