OPEN PEER REVIEW

Open Peer Review (OPR)

As a new feature for progressing towards transparency, we decided to open a new window for all of our editors as well as authors titled "**Open Peer Review**". We hope by this new facility, our reviewers will be more motivated and authors will be more satisfied with the review process. We believe that publishing our peer review reports could make a transparent and clear environment for all our efforts within a journal, but not all reviewers tend to publish their comments.

What is "Open Peer Review" process?

An "Open Peer Review" process is making the details of all review process (including reviewers, associate editors, and EICs comments) as "Public" as it is agreed by EIC, Authors, and reviewers.

Advantages of "Open Peer Review" process

- More transparency, constructiveness and tactful comments of the peer review process: leads to an increase in the quality of reviews
- More motivations for all involved roles in the review process
- Authors' satisfactions from the review process: Increases honesty between authors and reviewers
- Education of both authors and new students
- Prevents reviewers from following their individual agendas and leading to the detection of reviewers' conflicts of interests

You can find out more at:
https://sites.kowsarpub.com/kowsar/knowledgebase/category/tree.html#opr.html

Jundishapur Journal of Natural Pharmaceutical Products
Article DOI: 10.5812/jjnpp.101797
Published in: Jundishapur Journal of Natural Pharmaceutical Products: 16(1); e101797

Peer Review Report for "Evaluation of Cinnamon (Cinnamomum Verum) Effects on Liver CYP450 2D1 Activity and Hepatic Clearance in Diabetic Rats"

Author(s): Navid Neyshaburinezhad, Mohammadreza Rouini, Hoda Lavasani, Yalda H. Ardakani

Review Timeline:

Submit Date: 15 Feb 2020
Revised Date: 18 May 2020
Accepted Date: 28 Jun 2020

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

Mohammad Taghi Goodarzi: Reviewer | Revision (0) 25 Feb 2020

Dear author;

The manuscript contains new findings and properly designed and written; however needs some revisions.

1- All numbers in the text need "unit" ; just as an example : .....group (543 ± 27) (p-value = 0.00009.

2- The characteristics of HPLC must be described: The brand of machine, flow rate, detector , eluents....

3- It is not mentioned what factor was measured by HPLC.

4- In most figures the symbols (* **) inside Fig are different with those on legends; ** is not in Fig 3.

5- 300 µm this is micrometer ; it must be µM

6- What was the principle of CYP.. assay? the unit?

Good Luck!

REFEREE: Reviewer | **Revision (0)** 27 Mar 2020

Dear author;
Manuscript has been designed to investigate the effect of Cinnamon on Liver clearance/Biotransformation capacity in Diabetes type 1 & 2.
Overall, the manuscript is poorly written with remarkable irregularity in the context. So, I think that authors should re-write the manuscript from start to the end, point by point on the basis of an article writing manual. Then, after re-submission, it would be reviewed.

As clarification for the authors, some of comments are as the following:
1) The purpose of the study is not clear (last paragraph/introduction).
2) In introduction: Why two types of diabetes are described in the same way? Type 2 is a chronic disease and type 1 almost often is an acute and sudden one. So, complications are different as their therapies.
3. Introduction and discussion sections deserve further attention. Discussion is not designated for expressing the results again. Discussion would be much more interesting with adding and comparing the results of existing literature with current study and how the strategy differs from others.
4. The work will be more attracting if authors use English proof reading service to brush up their English expression.
5. Results have not been expressed as needed.

Good Luck in your future studies!

REFEREE: Reviewer | **Revision (0)** 26 Apr 2020

Dear Editor in Chief;

The article entitled “Evaluation of cinnamon (Cinnamomum verum) effects on liver CYP450 2D1 activity and hepatic clearance in diabetic rats." was read and the following comments
should be accomplished by the authors;

1. In abstract and results: Why are the mean values different between control group and other groups (High variations)?
2. In the end of introduction you must write the purpose of your study without any references.
3. How did you choose dose of cinnamon and other drugs in this study?
4. Did you use paired t test or student t test?
5. Did you assay CYP450 2D1 activity or concentration?
6. I think it is better authors assay liver function tests such as ALT ,AST,ALP and….

7. I think in discussion you should discuss CYP450 role in diabetes and notice molecular and cellular mechanisms of liver damage in diabetic patients.

Good Luck!

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

May 17, 2020

Dear Prof. Ali Asghar Hemmati,

Editor in chief, Jundishapur Journal of Natural Pharmaceutical Products,

Please find attached our revised manuscript entitled “Evaluation of cinnamon (Cinnamomum verum) effects on liver CYP450 2D1 activity and hepatic clearance in diabetic rats" ID: 101797. We are very pleased to have this opportunity to revise our manuscript according to all the suggestions of the reviewers. Below we have provided a point by point response to reviewer’s comments and yellow highlighted text indicates where changes have been made in the revised manuscript.

Reviewer 1:

Dear Editor in Chief

This article entitled “Evaluation of cinnamon (Cinnamomum verum) effects on liver CYP450 2D1 activity and hepatic clearance in diabetic rats.

Question 1: In abstract and results why is different between means in control group and other groups? High variation?

Answer1: The control group consists of **healthy non-diabetic rats** that have considered in this study to determine **the normal activity of CYP2D1** to assess any changes in the observed activity due to various factors such as disease states and so forth.

In other words, observing any changes in the calculated metabolic ratios compared to the observed value in healthy rats will be considered as an indicator of change in CYP2D1 activity in other treated groups.

Question 2: In the end of introduction you must write the purpose of your study without any references.

“In this study, changes in the ratio of dextrorphan (DXO) to dextromethorphan (DM) as an accepted probe for assessing the rat CYP2D1 were measured in order to examine the posttreatment effects of cinnamon consumption with or without insulin or metformin on CYP2D1activity and hepatic clearance were examined in type 1 and 2 diabetic rat perfused liver model [14].”

Answer 2: The above-mentioned part changed to: “It should be noted that the two most important types of diabetes were selected to investigate the effect of acute and chronic inflammatory conditions on the activity of CYP enzymes. In this study, any changes in the metabolic ratio of dextromethorphan (DXO) was considered as an index to evaluate the effects of cinnamon consumption with or without routine treatments of both types of diabetes on the CYP2D1 activity and the hepatic clearance parameters in a rat perfused liver model.”

Question 3: How did you choose dose of cinnamon and other drugs in this study?

Answer 3:

The dose of cinnamon was selected according to the literature review. The references were added, and the sentence was mentioned in the manuscript: "The dose of cinnamon was selected based on previous reported studies on diabetic rats [1, 2]."

Question 4: Did you use paired t test or student t test?

Answer 4: As we examined our hypothesis in different groups of rats, unpaired t-test was used for statistical analysis. It also has been mentioned in **"Statistical analysis"** part of the manuscript.

Question 5: Did you assay cyp450 2D1 activity or concentration?

Answer 5: This explanation phrase was added to the manuscript: **"**This study has been designed just to investigate on the changes in the CYP2D1 activity by calculating the metabolic ratio of dextromethorphan as an accepted probe for this type of studies, independent to the mechanisms of the observed events. Further studies are needed to investigate whether the expression of the enzyme was altered or NOT."

Question 6: I think it is the better authors assay liver function such as ALT, AST, ALP and….

Answer 6: We have assayed ALT and AST in order to monitor the viability of liver during perfusion process. It was not mentioned in the manuscript but now we added this explanation in the **"Methods part":** "Liver transaminases activities (AST and ALT) were also continuously monitored by a spectrophotometric method used as a measure of liver viability as it has been described by Kebis, A. et al. [3]."

Question 7: I think in discussion you should discuss cyp450 role in diabetic disease and notice molecular and cellular mechanisms of liver damage in diabetic patients.

Answer 7: As we mentioned in Question 5, we cannot discuss about the possible mechanisms, since experiments need to be done for this reason. On the other hand, this study

just reports the alternations in the enzyme activity which could be so important for dose adjustment in patient, and further researches is needed to understand the possible mechanism. We aimed to assess the effect of an inflammatory disease (diabetes mellitus in this case) on the metabolism process of the liver (CYP2D1 enzyme activity in this manuscript), as it has been previously demonstrated that inflammatory disease can alter metabolism capacity through “Phenoconversion” [4] of CYP450 enzymes (phenotype alterations) without considering the changes in expression or concentration of these enzyme (genotype alterations).

Reviewer 2:

Manuscript has been designed to investigate the effect of Cinnamon on Liver clearance/Biotransformation capacity in Diabetes type 1 & 2. At overall, the manuscript is poor written with remarkably irregularity in the context. So, I think that authors should re-write the manuscript from start to the end, point by point on the basis of an article writing manual. Then, after resubmission, it would be reviewed. For clarification to the authors, some of comments are as the following:

Question 1: The purpose of the study is not clear and concise (last paragraph/introduction)

Answer 1: As it has been previously demonstrated [4] that inflammatory disease can alter metabolism capacity through "Phenoconversion" of CYP450 enzymes (phenotype alterations) without considering the changes in expression or concentration of these enzyme (genotype alterations). So, we have changed the last paragraph of the "Introduction" in order to clarify the aim of our study: "It should be noted that the two most important types of diabetes were selected to investigate the effect of acute and chronic inflammatory conditions on the activity of CYP enzymes. In this study, any changes in the metabolic ratio of dextromethorphan (DXO) was considered as an index to evaluate the effects of cinnamon consumption with or without routine treatments of both types of diabetes on the CYP2D1 activity and the hepatic clearance parameters in a rat perfused liver model."

Question 2: In introduction, why two types of diabetes are used is not clear. Because type 2 is a chronic disease and type 1 almost often is an acute and sudden one. So, complications are different as their therapies.

Answer 2: Precisely, "we selected two important types of diabetes to investigate the effect of acute and chronic inflammatory conditions on the activity of CYP enzymes." As mentioned by the esteemed reviewer, these two inflammatory states could affect the body by different mechanisms. Therefore, we tried to highlight this important issue in the "**Introduction part**" of the manuscript: "It should be noted that the two most important types of diabetes were selected to investigate the effect of acute and chronic inflammatory conditions on the activity of CYP enzymes. In this study, any changes in the metabolic ratio of dextromethorphan

(DXO) was considered as an index to evaluate the effects of cinnamon consumption with or without routine treatments of both types of diabetes on the CYP2D1 activity and the hepatic clearance parameters in a rat perfused liver model."

Question 3: How CYP2D6 could involve in 20% biotransformation of drugs and etc.

Answer 3: It has been stated in several studies that CYP2D6 enzyme involves in metabolism of 20% of medicines [5, 6]. The refences for this claim have been inserted in the manuscript.

Question 4: Expressing of needed statements according to the purpose of the study deserves further attention to the introduction and discussion Sections. Discussion is not for expressing the results again, but discussion would be much more interesting with added the existing literature on how the strategy differs from others.

Answer 4: "According to the literature review, this is the first study, focusing on the benefits of cinnamon consumption on the activity of liver CYP enzymes. Although a lot of studies approved the hypoglycemic and hypolipidemic effects of different extracts of cinnamon, no similar investigation was found." We tried our best to add this concept to the discussion.

Question 5: The work will be more attracting if authors use English proof-reading service to brush up their English expression.

Answer 5: The manuscript has been edited by an English language expert and the certificate of service has been attached here.



CERTIFICATE

To whom it may concern,

This is to certify that paper/document below has been proofread by experts in the field of English language at Tarjomic translation agency.

Title: **Evaluation of cinnamon (Cinnamomum verum) effects on liver CYP۴۵۰ ۲D۱ activity and hepatic clearance in diabetic rats**

Author(s): **Navid Neyshaburinezhad, Mohammadreza Rouini, Hoda Lavasani, and Yalda Hosseinzadeh Ardakani**

Order No: **46176**
Translator/editor ID: **1919**
Verification code: **TRJ-Hxuges**
Date of completion: **May 9 , 2020**

Amir Bidokhti
Managing Director of Tarjomic

ترجمیک

This certificate can be verified at **tarjomic.com/cert/TRJ-Hxuges**

Question 6: Results have not been expressed as needed.

Answer 6: Unfortunately, the question is not clear and did not mention why the results are not enough. It would be appreciated helping us in this regard.

Reviewer 3: The manuscript contains new finding and properly designed and written; however, needs some revision.

Question 1: All numbers in the text need "unit"; just as an example: group (543 ± 27) (p-value = 0.00009.

Answer 1: All needed units (mg/dl for FBS and ml/min for Clearance) have been rechecked and added to the manuscript, except for the “Metabolic ratio” that is unitless.

Question 2: The characteristics of HPLC must be described: The brand of machine, flow rate, detector, eluents....

Answer 2: As the respected reviewer asked, we have added an explanation of method in the mentioned part: “The chromatographic apparatus consisted of a low-pressure gradient HPLC pump coupled with a fluorescence detector (320 excitation- 285 emission nm), a 100 µlit loop, and a Rheodyne model 7725i injector, all from Knauer (Berlin, Germany). A ChromolithTM Performance RP-18e 100 mm×4.6 column coupled with ChromolithTM guard cartridge RP- 18e 5 mm × 4.6 mm were applied for chromatographic separation (Merck, Darmstadt, Germany). A mixture of 20% methanol, 30% acetonitrile and 50% KH2PO4 buffer (10 mM, 0.02% of TEA, and pH 3.5) was employed as mobile phase and was passed through the system with a constant flow rate of 1 ml/min. Data acquisition and analyses were achieved by ChromGate chromatography software (Knauer, Berlin, Germany). Using this method, the retention time of dextrorphan and dextromethorphan were measured to be 5.5 and 10 min, respectively [7].”

Question 3: It is not mentioned what factor was measured by HPLC.

Answer 3: Although we mentioned in the “Pharmacokinetic parameters” that we used the concentrations of DOX and its metabolite, we added this issue in the “**Apparatus and chromatographic condition “**for more clarification and based on the esteemed reviewer comment.

"The areas under the concentration versus time curves $AUC0_{(0-60)}$ were computed using the trapezoidal rule based on concentrations of dextromethorphan and its metabolite that has been obtained by mentioned HPLC-fluorescence method."

Question 4: In most figures the symbols (***) inside Fig are different with those on legends; Fig 3 ** is not in Fig

Answer 4: It was corrected.

Question 5: 300 µm this is micrometer; it must be µM

Answer 5: It was corrected.

Question 6: What was the principle of CYP. assay? the unit?

Answer 6: As it is mentioned in the manuscript, any changes in the activity of the CYPs was assessed based on the changes in the metabolic ratio of DEX which is highly accepted as a probe to investigate the activity of CYP2D1 in rat that is the orthologue of CYP2D6 in humans. So, as we used the **ratios** (AUC of metabolite to the AUC of parent compound), no unit was mentioned.

References:

[1] Mohamed Sham Shihabudeen H, Hansi Priscilla D, Thirumurugan K. Cinnamon extract inhibits alpha-glucosidase activity and dampens postprandial glucose excursion in diabetic rats. Nutr Metab (Lond) 2011;8(1):46.

[2] Qin B, Nagasaki M, Ren M, Bajotto G, Oshida Y, Sato Y. Cinnamon extract (traditional herb) potentiates in vivo insulin-regulated glucose utilization via enhancing insulin signaling in rats. Diabetes Res Clin Pract 2003;62(3):139-48.

[3] Kebis A, Kukan M, Grancic P, Jakubovsky J. A novel way of liver preservation improves rat liver viability upon reperfusion. J Zhejiang Univ Sci B 2007;8(5):289-95.

[4] Shah RR, Smith RL. Inflammation-induced phenoconversion of polymorphic drug metabolizing enzymes: hypothesis with implications for personalized medicine. Drug Metab Dispos 2015;43(3):400-10.

[5] Owen RP, Sangkuhl K, Klein TE, Altman RB. Cytochrome P450 2D6. Pharmacogenet Genomics 2009;19(7):559-62.

[6] Zhou SF, Liu JP, Chowbay B. Polymorphism of human cytochrome P450 enzymes and its clinical impact. Drug Metab Rev 2009;41(2):89-295.

[7] Neyshaburinezhad N, Seidabadi M, Rouini M, Lavasani H, Foroumadi A, Ardakani YH. Evaluation of hepatic CYP2D1 activity and hepatic clearance in type I and type II diabetic rat models, before and after treatment with insulin and metformin. Daru 2020.

OPEN PEER REVIEW

Revision (1)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | **Revision** (1)

15 Jun 2020

Dear editor in chief;
This article has been revised and it is suitable for publication in this journal.

Sincerely yours!