Author's response to reviews

Title: Catechin prevents the calcium oxalate monohydrate induced renal calcium crystallization in NRK-52E cells and the ethylene glycol induced renal stone formation in rat

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Author's response to reviews:

Dear Editor:

The manuscript 6719926469494195 entitled “Catechin prevents the calcium oxalate monohydrate induced renal calcium crystallization in NRK-52E cells and the ethylene glycol induced renal stone formation in rat” has been revised according to the comments and suggestions. And we very appreciate the useful and valuable suggestions from you and reviewers. The changes in the revised manuscript are highlighted. We would like to resubmit this revised manuscript to BioMed Central, and hope it is acceptable for publication in the journal. Please do not hesitate to contact us with additional questions or concerns.

Answers to reviewers’ questions were as follows:

Reviewer 1 (Juan-Jose Ventura):

The present study reports the catechin prevents the calcium oxalate monohydrate induced renal calcium crystallization by in vitro and in vivo experiments and the manuscript contains new and significant information. However, there are some shortcomings to be recomposed.

1. The language needs polishing so that the goals and results of the study are clear to the reader.
   
   Answer: Thanks very much for your comments. Our manuscript has been revised with the help of native speaker regarding the deficiencies in English grammar, spelling, and sentence structure.

2. All abbreviations must be defined at their first mention there.
   
   Answer: Thanks very much for your comments. We have checked our manuscript thoroughly and all abbreviations have been defined when they occurred at the first time.

3. Abstract: The in vitro and in vivo experiments are not described clearly.
   
   Answer: Thanks very much for your comments. The in vitro and in vivo
The works authors have done are interesting and important and I suggest it be accepted by this journal. However, this manuscript needs to be revised before being accepted.

1. All abbreviations must be defined at their first mention there.

Answer: Thanks very much for your comments. We have checked our manuscript thoroughly and all abbreviations have been defined when they occurred at the first time.

2. Abstract: You should indicate in vitro and in vivo experiments.

Answer: Thanks very much for your comments. The in vitro and in vivo experiments in the abstract have been reconstructed for better understanding as follows:

In the vitro experiment, the changes of the mitochondrial membrane potential, expression of superoxide dismutase (SOD), 4-hydroxynonenal (4-HNE), cytochrome c, and cleaved caspase 3 were measured to show the effects of catechin treatment on the NRK-52E cells induced by calcium oxalate monohydrate (COM). In the vivo study, Sprague-Dawley rats were administered 1% ethylene glycol (EG) to generate a rat kidney stone model and then treated with catechin (2.5 and 10 mg/kg/day) for 14 days. The urine and serum variables were detected on 7 and 14 days after EG administration. The expression of cytochrome c, cleaved caspase 3, SOD, osteopontin (OPN), malondialdehyde (MDA), 8-hydroxy-2'-deoxyguanosine (8-OHdG), cytochrome c and cleaved caspase 3 in kidney were measured. Furthermore, the mitochondrial microstructure in the kidney was also examined by transmission electron microscopy.

4. Please provide more details about significant difference in Fig 5 and Fig 6.

Answer: Thanks very much for your comments. We have provided more details about significant difference in Fig 5 and Fig 6.

Figure 5: The ratios of the OPN, MDA and 8-OHdG expression areas were significantly higher in the EG group than those in the other groups (P<0.05, Figure 5). Meanwhile, there was no significant difference in the ratios of SOD, OPN, MDA and 8-OHdG expression areas among the control group, EG + catechin 2.5 group and EG + catechin 10.0 group (P>0.05, Figure 5).

Figure 6: In EG + catechin 2.5 group and EG + catechin10.0 group, the expression of OPN was higher than that in EG group (P<0.05). There was a significant change between the control and EG group. A significant difference between EG group and EG + catechin groups was also observed (P<0.05). The expression of cytochrome c had no changes among the four groups. There was a significant change in cleaved caspase 3 between the control and EG + catechin 2.5 group (P<0.05). There were no significant differences among the other three groups for the expression of cleaved caspase 3 in crystal-model rat kidneys (P>0.05).
follows:

In the vitro experiment, the changes of the mitochondrial membrane potential, expression of superoxide dismutase (SOD), 4-hydroxynonenal (4-HNE), cytochrome c, and cleaved caspase 3 were measured to show the effects of catechin treatment on the NRK-52E cells induced by calcium oxalate monohydrate (COM). In the vivo study, Sprague-Dawley rats were administered 1% ethylene glycol (EG) to generate a rat kidney stone model and then treated with catechin (2.5 and 10 mg/kg/day) for 14 days. The urine and serum variables were detected on 7 and 14 days after EG administration. The expression of cytochrome c, cleaved caspase 3, SOD, osteopontin (OPN), malondialdehyde (MDA), 8-hydroxy-2'-deoxyguanosine (8-OHdG), cytochrome c and cleaved caspase 3 in kidney were measured. Furthermore, the mitochondrial microstructure in the kidney was also examined by transmission electron microscopy.

3. Please provide the method of cell counting.

Answer: Thanks very much for your comments. In the method of cell culture, cell suspension was prepared and counted in the blood counting chamber. And we have supplemented the method of cell counting in the method of cell culture.

Reviewer 3 (Vittorio Fineschi):

Minor essential revisions

The paper is interesting and deserves to be published. I have only few comments.

Comment 1: The paper is well argumented, the methods are appropriate and well described, but it should be useful for the readers to introduce in the section titled "Ultrastructural findings of kidneys exposed to EG in the control and crystal-model rat", the concepts about COM internalization by HPT cells. So, figure 7 needs to be expanded by a detailed legend about the morphology of the picture shown.

Answer: Thanks very much for your comments. The legend of Figure 7 has been supplemented with the morphology of the rat kidney sections as follows:

The renal tubules were circular, microvilli were evident in the lumen, and mitochondria were located around the nuclei in the control group. In the EG group, the renal tubules were thin with flattened tubular cells; the lumen of the renal tubule was expanded; microvilli were barely recognizable; crystals were present in the lumen; swollen mitochondria resembling fat droplets around the nuclei had an indistinct, discontinuous, and partly collapsed double membrane. In the EG + catechin (EG + catechin 2.5, and EG + catechin 10.0) groups, the renal tubules were circular and microvilli were detected in the lumen; the renal tubules were longer than those in the EG group, and the layer was thicker, but slightly shorter than that in the control group; the mitochondria had a regular internal structure with a continuous double membrane which was similar to the mitochondria in the control group.

Comment 2: Again, it would be interesting to specify the role of calcium.

Literature results also stress the influx of extracellular calcium to produce toxicity
and oxidative stress to renal cells.

Answer: Thanks very much for your comments. We have supplemented some references to introduce the toxicity and oxidative stress to renal cells produced by extracellular calcium in the introduction, as follows.

It has already known that exposure to high levels of oxalate and calcium oxalate crystals can induce oxidative stress such as an increase in free radical generation, increased lipid peroxidation, a decrease in cellular anti-oxidant status and an increase in phospholipase-A2 (PLA2)-induced release of arachidonic acid [1-3]. Sustained exposure to high levels of oxalate or calcium oxalate crystals injures the cells [4]. Mitochondria have been demonstrated to show excessive uptake of calcium when the cytoplasm level of free calcium markedly increases, causing abnormalities in the respiratory chain and increasing the mitochondrial production of ROS [5, 6]. Calcium-induced mitochondrial injury can be prevented by antioxidants suggesting that oxidative stress may be an important event in its development [5].

Comment 3: Legend of figure 4, needs to be expanded with a clear explanation about the reactions and the results of immunohistochemistry.

Answer: Thanks very much for your comments. We have expanded the legend of Figure 4 with a clear explanation about the reactions and the results of immunohistochemistry as follows:

Strong expression of SOD was could be observed in the control group, but SOD was not detected in EG group. The expression of SOD was slightly lower in EG + catechin 2.5 group than that in EG+catechin10.0 group. OPN was barely detectable in the control group, but it was strongly expressed in the EG group. The OPN expression in EG + catechin2.5 group was slightly higher than that in EG + catechin10.0 group. MDA was undetectable in the control group and EG + catechin groups, but it was detectable in EG group kidneys. The expression of 8-OHdG was undetectable in the control group, but it was detectable in the EG group. And the expression of 8-OHdG was nearly undetectable in the EG + catechin groups.

Comment 4: Authors must cite some relevant contributions about ROS and renal toxicity in the references list.

Answer: Thanks very much for your comments. We have supplemented some references about ROS and renal toxicity in the introduction as follows:

Renal toxicity is assumed to be caused by the elevation of serum-free iron concentration, following its reduction at the luminal side of the proximal tubule, which generates ROS decreasing antioxidant systems and also leads to the enhancement in lipid peroxidation [7, 8]. It has been demonstrate that toxic action of acephate on kidney cells is partly through an ROS-mediated mechanism[9]. And ROS are known to mediate many toxin induced renal tubular injuries [10-12].

Reviewer 4 (SUHAILA MOHAMED):

'Catechin prevents the calcium oxalate monohydrate induced renal calcium crystallization in NRK-52E cells and the ethylene glycol induced renal stone formation in rat'
This work is quite good, well designed and executed, and fairly well written for authors whose mother tongue is not English.

In assessing the work, the following points were considered:

1. The question posed by the authors was well defined.
   Answer: Thanks very much for your affirmation and encouragement.

2. The methods were appropriate and well described.
   Answer: Thanks very much for your encouragement.

3. The data was sound.
   Answer: Thanks very much for your comments.

4. The manuscript adhered fairly well to the relevant standards for reporting and data deposition.
   Answer: Thanks very much for your comments.

5. The discussion and conclusions was well balanced and adequately supported by the data. However some explanations on the choice of the parameters monitored could be added to educate/assist new researchers in this field.
   Answer: Thanks very much for your comments. In our study, the changes of the mitochondrial membrane potential, expression of superoxide dismutase (SOD), 4-hydroxynonenal (4-HNE), cytochrome c, and cleaved caspase 3 were measured in vitro. On the other hand, The expression of cytochrome c, cleaved caspase 3, SOD, osteopontin (OPN), malondialdehyde (MDA), and 8-hydroxy-2’-deoxyguanosine (8-OHdG) in kidney were measured. Some parameters have been explained in the discussion, such as OPN, 4-HNE, SOD, 8-OHdG, MDA. Therefore, we have supplemented some explanations on the choice of the parameters including mitochondrial membrane potential, cytochrome c, and cleaved caspase 3 monitored in the introduction as follows:

In renal tubular cell injury, mitochondrial damage has been recognized as a crucial cause for tubular cell death which involves disruption of respiration complexes and loss of mitochondrial membrane potential [13, 14]. And cell apoptosis is precipitated by mitochondrial outer membrane permeabilization and consequent release of apoptogenic factors such as cytochrome c [15]. Caspases are crucial mediators of programmed cell death (apoptosis). Among them, caspase-3 is a frequently activated death protease, catalyzing the specific cleavage of many key cellular proteins[16]. Caspase-3 is activated in the apoptotic cell both by extrinsic (death ligand) and intrinsic (mitochondrial) pathways[17, 18]. In intrinsic activation, cytochrome c from the mitochondria works in combination with caspase-9, apoptosis-activating factor 1 (Apaf-1), and ATP to process procaspase-3[16, 19, 20].

6. The limitation of the work was not clearly stated.
   Answer: Thanks very much for your comments. The limitations of the work have been clearly stated in the discussion as follows:

In conclusion, the results of our study have showed that catechin have preventive effects on renal calcium crystallization both in vivo and in vitro. However, the
specific molecular mechanisms of catechin in renal calcium crystallization need to be further studied.

7. The authors did acknowledge work upon which they are building.
Answer: Thanks very much for your comments.

8. The title and abstract conveyed what has been found.
Answer: Thanks very much for your comments.

9. The writing requires some minor editing, as to spelling, some sentence construction and grammar and some terminologies (e.g. positive and negative controls may be confused).
Answer: Thanks very much for your comments. Our manuscript has been revised with the help of native speaker regarding the deficiencies in English grammar, spelling, and sentence structure.

I believe the research constitutes a useful and possibly significant contribution to the field and be considered for publication.

We appreciate very much for your time in reviewing our manuscript. And the suggestions and comments really helped us a lot. If there is any information I can provide, please don’t hesitate to contact us.

Thank you again for your time and patience. We are looking forward to hearing from you soon.

With kindest regards to you.

Yours sincerely,

Yun-Fei Xu

References


