Author's response to reviews

Title: Antioxidant effect of gallic acid from Phyllanthus emblica extract prevents contrast-induced acute kidney injury

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Dear the Editor,

We would like to express our appreciation to the Editor and the reviewers for all thoughtful suggestions. We have replied all the questions and comments. We have added and corrected following the advices from the reviewers in the new version of the manuscript. All the added or rewritten sentences are red-colored. We do hope that, now, the manuscript would be accepted for publication in the journal.

Yours sincerely,
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Itemized changes and Specific comments to the reviewers

Thank you very much for your useful comments. According to the reviewers’ comments, we have made several revisions to improve this manuscript. All the added or rewritten sentences are red-colored.

Response to editor

- We already clarify and change the format of Table and Figure.
- We already clarify and change the format in Authors’ contributions.
We clarify and add the detail of PE extract in the Methods-Sample “Phyllantus embelica fruits collected from Ampor Marim, Chiengmai Province Thailand on December 2011, were dried by oven at 50 °C. Dry plant material (10 kg) was grinded and boiled in water for 30 min, filtered and evaporated by evaporator. The extract was dried by freeze dry as water extract of PE (PEW). The percentage of yield obtained as 40.81%. The samples have been preserved in the refrigerator (-20 oC). Authentication of plant materials was identified by comparing against the specimens deposited at the herbarium of Southern Center of Thai Medicinal Plants, Faculty of Pharmaceutical Science, Prince of Songkla University, Songkla, Thailand, where herbarium vouchers have been kept (Herbarium no.SKP 071160501)”.

Response to reviewer 1: Zilong Li

1. We add the reference and compare the results of the antioxidant effect of vitamin E studies from our group as the positive control in discussion “The dose dependent effect of PE extract started at dose 250 mg/kg/d and had the additional effect at dose 500 mg/kg/d similar to the antioxidant effect of vitamin E in the experimental study [21] and clinical trials [25, 40].”


2. We add the reference for standard score of histopathological changes in the kidney.


3. We already put it on Figure legends.

Figure 3 Histopathological with H& E staining of CI-AKI in rats
A and B show normal rat kidneys in the cortex and medulla. Rats induced with CM (C and D) demonstrate severe TEC necrosis, moderate PTC congestion (black arrows) and proteinaceous casts (green arrows) in the cortex and medulla. Rats induced with CM and pre-treatment with PE extract 250mg/kg/day (E and F)
show a decrease in the severity of TEC necrosis, PTC congestion, and proteinaceous casts in the cortex and medulla. Interestingly, rats induced with CM and pre-treatment with PE extract 500mg/kg/day (G and H) demonstrate minimal TEC necrosis, PTC congestion or proteinaceous casts in the cortex and medulla, similar to control-rat kidneys. (Magnifications: x400 in A-H).

CM: contrast media; PTC: peritubular capillaries; TEC: tubular epithelial cells.

4. We agree with the reviewer but we have not done these examinations. So, we discuss this situation in Study limitations “Monitoring of urine output and/or novel biomarker in plasma or urine is a much better indicator for early diagnosis of CI-AKI. However, we have measured only serum BUN and Cr levels. Also, we have only H&E and PAS staining for histopathologic examination of renal tissues. Therefore, the immunohistochemistry staining such as Tunnel or PCNA and the evaluation of protein and gene expression in apoptosis or inflammatory pathway should be prepared to confirm CI-AKI”.

Response to reviewer 2: Ajith A.TH. T.A

1. We change the title to “Antioxidant effect of Phyllanthus emblica extract prevents contrast-induced acute kidney injury”.

2. -No study try to use PE extract prevent CI-AKI before.
   -Only chronic renal impairment, but not diabetes mellitus is the risk factor of CI-AKI.
   -We choose this model to induce CI-AKI because this experiment uses indomethacin for inhibit the prostaglandin synthesis and L-NAME for inhibit the nitric oxide synthesis before contrast media administration. Many studies accept and use this model.


3. We try to find the appropriate dose by using the double dose in each group and may be can apply to the clinical trial.

4. We agree with you but different technique for PE extraction could separate the different compound from PE.

5. The dosage of the CM that used in this study follows the protocol (reference above).

6. The serum BUN and creatinine levels in CM+ PE extract at doses of 250 and 500 mg/kg/day were close to the normal. However, the histopathological analysis in CM+PE extract at doses of 500 mg/kg/day was better than CM+ PE extract at
doses of 250 mg/kg/day.

7. We clarify and add “All measurements were performed using standard methods in a single, hospital-based laboratory [25]” in Methods-Biochemical assay.


8. We already have the control compare with CM group that receive PE extract at doses of 125, 250 and 500 mg/kg/day.

Response to reviewer 3: Omer Toprak

1. Thank you very much for your kindness.

2. We change “These findings suggest that pretreatment with PE extract provides the renoprotective effect against CI-AKI in rat model and needs further development to assess its clinical usefulness” to “This study demonstrated the protective role of PE extract against CI-AKI” in abstract-conclusion section.

3. We add the reference and clarify the rationale for 5 day pre-treatment of PE extract in 6th paragraph of Discussion.

The plasma levels of PE extract and the accumulation of PE extract on renal tissues with the repeated daily dose may be higher than the single dose administration. In experimental study, the bioavailability of gallic acid from grape seed polyphenol extract is improved by repeated dosing in rats [52]. The single dose with oral administration demonstrated that the intestinal absorption of gallic acid is poor (<2%). While repeated exposure to the extract has shown the absorption of gallic acid was significantly higher than single dose treatment and reached to tissues level at day 10. Moreover, two gallic acid-derived compounds isolated from Casearia sylvestris leaves could reverse NK cell cytolysis which was suppressed from tumor growth when mice were treated with these compounds for 4 days [53]. In contrast, a single dose administration with a large volume and high concentration of any compound via oral route may cause adverse effects and could not reach to the therapeutic levels in the tissues. From these informations, we make the decision to give PE extract orally as presented in the protocol. However, a high dose administration of PE extract for 2 or 3 days before contrast administration should be evaluated for the preventing CI-AKI.


4. We add the “Study limitations” section to the discussion before the conclusion.

Study limitations
Monitoring of urine output and/or novel biomarker in plasma or urine is a much better indicator for early diagnosis of CI-AKI. However, we have measured only serum BUN and Cr levels. Also, we have only H&E and PAS staining for histopathologic examination of renal tissues. Therefore, the immunohistochemistry staining such as Tunnel or PCNA and the evaluation of protein and gene expression in apoptosis or inflammatory pathway should be prepared to confirm CI-AKI.