

Reviewer #1:

Thanks for your detailed examination and I will resolve them one by one.

1. Excellent study on the possible protective role of myricetin in DM induced Nephropathy.

Thanks for your recognition of our research.

2. The authors must incorporate an Algorithm on possible mechanism of action of Myricetin.

I have added and made some change in the part of INTRODUCTION, the following is the changed:

Abelmoschus manihot capsule, an important Chinese patent medicine and widely used in treating kidney diseases, consists of seven flavonoids, including rutin, hyperoside, quercetin, myricetin, hibifolin, isoquercetin, and quercetin-3-o-robinobioside^[22]. Our studies have been devoted to examining the roles of the total flavones of *Abelmoschus manihot*^[23], hyperoside^[24] and quercetin^[25] in animal models of DN. Myricetin, present in dicotyledonous plants, has demonstrated a wide range of medicinal properties including anti-inflammatory, anti-cancer, and hepatoprotective^[26,27]. For example, Park *et al*^[28] found that 30 μ M of myricetin suppresses NF- κ B activation and attenuates the secretion of TNF- α and IL-6. Liao and colleagues^[29] found that myricetin prevented diabetic-associated cardiac injury in STZ-induced mice and in high glucose-challenged neonatal rat cardiomyocytes. These investigators also found that myricetin possesses a potential protective effect by inhibiting I κ B α /NF- κ B pathways and enhancing Nrf2/HO-1. Kandasamy and colleagues^[30] found that STZ-induced diabetic nephrotoxic rats treated with myricetin were protected from glomerular injury, further suggesting its potential as an anti-hyperglycemic agent. These data collectively indicate that myricetin has effects on inhibiting the secretion of inflammatory factors and its potential therapeutic functions on diabetic-related disease as well. However, the mechanism underlying how myricetin inhibits the progress of DN remains a mystery.

3. Any specific reason for doing testing on db/db mice RAW 264.7? Needs to be elaborated.

I have elaborated the corresponding context in introduction. The following is the added: Macrophages, famous for their pluripotency and plasticity, differentiate into classically activated (M1) cells and alternatively activated (M2) cells, playing opposing roles in the regulation of inflammation^[13]. M1 cells are closely associated with the proinflammatory response, and increased expressions of CD86, tumor necrosis factor-alpha (TNF- α) and inducible nitric oxide synthase (iNOS) represent the phenotype transformation of M1; whereas, M2 cells have increased expression of CD206, arginase-1 (Arg-1) and interleukin (IL)-10^[14,15]. More importantly, the above two distinct cell subsets exist in a dynamic balanced state. Indeed, one study has indicated that the regulation of

macrophage polarization could inhibit renal inflammation in mice with DM^[16].

4. How the dose of Myricetin was titrated? Needs to be mentioned.

I have added the related explanation in the parts of RESULTS.

As shown in Figure 6A, a concentration gradient of myricetin at 12.5 μ M, 25 μ M, and 50 μ M was administered to the cells for 24 h. Flow cytometry analysis revealed that 25 μ M myricetin exhibited the most potent inhibitory effect on M1-type polarization of the RAW 264.7 cells.

5. After staining at what magnification H/P Images were seen? Does all images were easily captured? Must be mentioned.

I have added some contents in the parts of METHODS.

Ten sections were chosen from every mouse and each was examined under light microscope at 100 $\times$ optical magnification. Histological changes were assessed at 200 $\times$ optical magnification. Brightfield images were acquired using an IX83 microscope (Olympus, Tokyo, Japan), and these images were subsequently analyzed using Image-Pro Plus software (Media Cybernetics, Rockville, MD, United States). Semi-quantitative analysis was performed to compare the samples from each group and a histogram was made. Representative renal images are presented from each group.

6. What was the principle behind uACR - calibration and standardisation? Not mentioned.

I have added some contents in the parts of METHODS.

Urinary albumin was measured by immunoturbidimetry and creatinine by the sarcosine oxidase method. The uACR measurement was calculated by dividing urinary albumin by urinary creatinine (μ g/mg), which could be applied for detection, diagnosis and monitoring. The calibration of urine albumin by creatinine can effectively avoid the interference of other baseline factors such as body weight (BW) and food intake, and ensure comparability of the results.

7. The authors must mention possible trials in humans in the discussion section.

To our knowledge, only one cross-sectional population clinical study (consisting of 24138 subjects, among which 1357 had type 2 DM) has shown that myricetin intake might lower the prevalence type 2 DM and extend the period until other clinical treatments become necessary^[47]. Other studies have shown that *Abelmoschus manihot* capsule containing myricetin could be useful in decreasing proteinuria, blood creatinine and blood urea nitrogen in kidney patients^[48,49].

8. Does Myricetin has any hypoglycaemic - action?

Yes, myricetin has the effects of hypoglycaemic-action and I have added in discussion. Detailed is shown as below:

More recently, it has been reported that kaempferol and myricetin combination treatment is promising in diabetes rats, due to their modulation of levels of glucose, inflammation, lipids and liver enzymes^[42]. Another study has further demonstrated that DM could be alleviated by myricetin alone *via* its effects on normalizing the profile of intestinal flora^[43].

9. Any possible protective role in other pathology caused by DM like - Neuropathy, Cardiopathy, Retinopathy etc must be added in the discussion part.

Yes, myricetin played protective in other diabetic complications and I have added in

discussion. Detailed is shown as below:

Importantly, myricetin not only plays a vital role in DN but also decreases migration of retinal pericytes^[44], restores impaired motor and sensory functions^[45], and enhances diabetic wound repair^[46].

Reviewer #2:

The article is well written with some minor grammatical and typographic mistakes.

Thanks for your approval and I have modified my article. Meanwhile, I will send it to the professional English editing company.