Morin Improves Urate Excretion and Kidney Function through Regulation of Renal Organic Ion Transporters in Hyperuricemic Mice

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ABSTRACT - Purpose. Morin (2′,3,4′,5,7-pentahydroxyflavone), a plant-derived flavonoid, has beneficial effects on hyperuricemia and renal dysfunction in animals. Since the decreased renal excretion of uric acid is the hallmark of hyperuricemia, here we studied the effects of oral morin administration on renal organic ion transporters in potassium oxonate-induced hyperuricemic mice. **Methods.** Hyperuricemia in mice was induced by potassium oxonate. Uric acid and creatinine concentrations in urine and serum, and fractional excretion of uric acid (FEUA) were performed to evaluate renal urate handling. Changes in expression levels of renal organic ion transporters were detected by Western blotting and semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) methods. **Results.** Morin treatment significantly increased urinary uric acid/creatinine ratio and FEUA, resulting in reduction of serum uric acid levels in hyperuricemic mice. And kidney conditions were also improved after morin treatment in this model. Protein and mRNA levels of glucose transporter 9 (mGLUT9) and urate transporter 1 (mURAT1) were significantly decreased, and of organic anion transporter 1 (mOAT1) were remarkably increased in the kidney of morin-treated hyperuricemic mice. Morin treatment also blocked down-regulations of renal organic cation and carnitine transporters (mOCT1, mOCT2, mOCTN1 and mOCTN2) in hyperuricemic mice. **Conclusion.** These results suggest that morin exhibits the uricosuric effects via suppressing urate reabsorption and promoting urate secretion in the kidney of hyperuricemic mice and may help to attenuate deleterious effects of hyperuricemia with renal dysfunction.

INTRODUCTION

Hyperuricemia has been associated with gout, hypertension, kidney disease and metabolic syndrome (1-4). The impaired renal uric acid excretion is the hallmark of hyperuricemia (5). Some of renal organic anion transporters such as glucose transporter 9 (GLUT9, encoded by *SLC2A9*), urate transporter 1 (URAT1, encoded by *SLC22A12*), organic anion transporters 1 (OAT1, encoded by *SLC22A6*) and 3 (OAT3, encoded by *SLC22A8*), are involved in mediating urate handling (6-11). Mutations in GLUT9 deficiency cause urate reabsorption reduction, resulting in the decreased serum uric acid levels and increased fractional excretion of uric acid (FEUA) (12). And the defect in hURAT1 is associated with the pathogenesis of primary hyperuricemia and gout (13, 14). In addition, OAT1-knockout mice show reduction of uric acid secretion (15). Down-regulations of renal rOAT1 and rOAT3 are detected in uricase inhibitor oxonic acid and uric acid-induced hyperuricemic rats (16, 17). These observations suggest that GLUT9, URAT1, OAT1 and OAT3 may play important roles in urate excretion impairment.

Hyperuricemia is known to cause renal dysfunction (1, 18). Renal tubular transport of organic cations is mediated by renal organic cation and carnitine transporters (OCTs and OCTNs) (19-22). The altered expressions of OCT1 (encoded by *SLC22A1*) and OCT2 (encoded by *SLC22A2*) at the basolateral membrane of renal tubules are related to renal function impairment. Laboratory evidences demonstrate down-regulations of renal rOCT2 protein and mRNA levels in oxonic acid-induced hyperuricemic rats (16, 17), and of renal rOCT2 protein levels in 5/6 nephrectomy rats with chronic renal failure (23). Streptozotocin-induced diabetes of rats with kidney dysfunction also shows reductions in protein levels of renal rOCT1 and rOCT2, and mRNA levels of renal rOCT2 (24). OCTN1 (encoded by *SLC22A4*) at the brush border membrane of renal tubules is a susceptibly factor related to the ethopathology of autoimmune disorders (25) and Crohn's disease (26). Defect mutations in hOCTN2 (encoded by *SLC22A5*) are suggested to cause the impaired reabsorption and secretion of organic cations (27).

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In addition, OAT1 alteration contributes to renal dysfunction in ischemia-reperfusion injured rats (28) and patients (29). These observations indicate that abnormality of these renal organic ion transporters may be related to renal dysfunction in hyperuricemia.

Hypouricemic drugs such as uricosuric agents and xanthine oxidase inhibitors are available. However, they have some of undesirable side effects, making their uses of limited value (30-33). Our recent research interest is to search for more effective agents for anti-hyperuricemic agents mediated by regulation of renal organic ion transporters (34, 35). Morin (2′,3,4′,5,7-pentahydroxyflavone) is abundant in twigs of *Morus alba* L., a diuretics for the treatment of hyperuricemia and gout in traditional Chinese medicine. Our early study reported that morin exhibited hypouricemic effect in hyperuricemic mice (36). However, this observed effect seemed not to parallel with its inhibitory activity of hepatic xanthine oxidase compared with that of xanthine oxidase inhibitor allopurinol, indicating that morin might exert uricousric effect by regulating renal urate handling apart from the enzyme activity inhibition simply. Recently, morin is confirmed to have uricosuric action in oxonate-induced hyperuricemic rats (37). Further evidences from i*n vitro* studies show that morin inhibit urate uptake in rat renal brush border membrane vesicles (37) and reduce urate uptake in human kidney cell system by hURAT1 (38). Morin is also beneficial to delay nephrotoxicity in Madin-Darby canine kidney cells via hOAT1 (39) and hOAT3 (40). However, the possible mechanism responsible for regulation of renal urate excretion and function by morin in hyperuricemia is not well understood. Urate transport in mouse kidney is similar to that in human kidney (41). Therefore, the objective of the present study was to investigate the effects of morin on expression levels of renal mGLUT9, mURAT1, mOAT1 and mOAT3, focusing on its urate excretion enhancement in potassium oxonate-induced hyperuricemic mice. Simultaneously, expression levels of renal mOCT1, mOCT2, mOCTN1 and mOCTN2 were determined to evaluate kidney function improvement of morin in this model.

METHODS

Chemical and reagents

Morin, allopurinol, uric acid and potassium oxonate were obtained from Sigma-Aldrich (St. Louis, MO). Creatinine assay kit was obtained from Jiancheng Biotech (Nanjing, P. R. China). Trizol reagent was purchased from Invitrogen Co, USA. Reverse transcriptase moloney murine leukemia virus (M-MLV) used for cDNA synthesis was from Promega Co, USA. Taq DNA polymerase and polymerase chain reaction (PCR) buffer mixture were from Sunshine Biotechinology Co. Ltd., P. R. China. Mouse glyceraldehyde-3-phosphate dehydrogenase (mGAPDH) was purchased from KangChen BIO-TECH (Shanghai, P. R. China). The antibodies, mGLUT9, mURAT1, mOAT1, mOAT3, mOCT1 and mOCT2 were purchased from SaiChi Biotech (Beijing, P. R. China). mOCTN1 and mOCTN2 were obtained from Alpha Diagnostic International Inc. (San Antonio, USA). Mouse Na^+-K^+-ATPase (mNa^+-K^+-ATPase) was purchased form Cell Signaling Technology, Inc. (Boston, MA, USA), and HRP-conjugated goat anti-rabbit or mouse IgG was obtained from Jingmei Biotech (Shanghai, P. R. China). All the primers (Table 1) were designed and synthesized by Sunshine Biotechinology Co. Ltd. (Nanjing, P. R. China). Other reagents were analytical grade made in P. R. China.

Animals

Male Kun-Ming strain of mice (20 ± 2 g) was purchased from Henan Experimental Animal Center (Henan Province, P. R. China). They were housed in plastic cages with free access to food and tap water at room temperature (22 ± 2 ^oC) with relative humidity (55 ± 5 %), under a normal 12-h/12-h light/dark schedule with the lights on at 08:00 a.m. They were allowed at least 1 week to adapt the environment before the experiment started. All studies were carried out in accordance with the Institutional Animal Care Committee and the China Council on Animal Care at Nanjing University.

Hyperuricemic mice and drug administration

Hyperuricemia in mice was induced by oral administration of 250 mg/kg potassium oxonate for seven consecutive days as our previous reports described (34, 35). Allopurinol (2.5 and 5 mg/kg) were used as a positive drug (34, 35). According to the previous report (37), four doses of 10, 20, 40 and 80 mg/kg morin were chosen to test for the possible mechanism of uricosuric effect and renal function improvement in hyperuricemic mice. Oxonate, morin and allopurinol were dissolved or dispersed in distilled water, respectively. All doses were expressed as mg/kg body weight of the respective drugs. The volume of drug administration was based on body weight measured immediately prior to each dose, respectively. Food, but not water, was withdrawn from the animals 1 h prior to the administration.

Table 1. Summary of gene-specific PCR primer sequences, length of production and the appropriate annealing temperature used in the experiments.

Description	Genebank	Sense primer (5'→ 3')	Antisense primer (5'→ 3')	Product size (bp)	Tm (°C)	Numbers of thermal cycle
mGLUT9	NM_001012363	CTCATTGTGGGACGGTTCA	GCTACTTCGTCCTCGGTA	316	56	30
mURAT1	NM_009203	GCTACCAGAATCGGCACGCT	CACCGGGAAGTCCACAATCC	342	58	30
mOAT1	NM_008766	ACGGGAAACAAGAAGAGGG	AAGAGAGGTATGGAGGGGTAG	580	56	29
mOAT3	NM_031194	TGCTACTGGCTTTGCCTACT	CTCCTGCTTTGTTTTCTTGG	556	56	29
mOCT1	NM_009202	ACATCCATGTTGCTCTTTCG	TTGCTCCATTATCCTTACCG	315	56	29
mOCT2	NM_013667	ACAGGTTTGGGCGGAAGT	CACCAGAAATAGAGCAGGAAG	331	56	29
mOCTN1	NM_019687	TGTTCTTCGTAGGCGTTCT	TGGAATAAACCACCACAGG	392	53.3	30
mOCTN2	NM_011396	TCTACGAAGCCTCAGTTGC	ATTCCTTTGACCCTTAGCAT	623	53.3	30
mGAPDH	NM_008084	TGAGGCCGGTGCTGAGTATGT	CAGTCTTCTGGGTGGCAGTGAT	299	58	35

Our preliminary experiment demonstrated that 80 mg/kg morin did not alter serum and urine biochemical indexes in normal mice (Data not shown). Therefore, mice were divided into normal group receiving water (normal-vehicle) and hyperuricemic groups receiving 250 mg/kg oxonate (hyperuricemia-vehicle), 10, 20, 40 and 80 mg/kg morin groups, 2.5 and 5 mg/kg allopurinol groups. Each group contained 8 mice. Oxonate or water was administered to mice in a volume of 10 ml/kg by gavage once daily at 9:00 a.m. for seven consecutive days. Morin and allopurinol were orally initiated at 10:00 a.m. on the day after oxonate was given, respectively.

Urate handling investigation

Each mouse in normal and hyperuicemic groups was housed in metabolic cages during the 6th day when animal had free access to standard chow and tap water. 24-h urine was collected and urine volume was recorded for each group, respectively. Urine samples were centrifuged at 2,000 × g for 10 min to remove the particulate contaminants and supernatant was used for uric acid and creatinine assays. After urine collection, tail-vein blood samples were collected, and then centrifuged (3,000 × g) at 4 °C for 10 min to get serum for uric acid and creatinine assays. Serum uric acid (Sur) and creatinine (Scr) concentrations, as well as urine uric acid (Uur) and creatinine (Ucr) concentrations were measured as below description, respectively. Fractional excretion of uric acid (FEUA) was calculated using the formula: FEUA = (Uur × Scr)/(Sur × Ucr) × 100, expressed as percentage (42).

Kidney sample collection

After urate handling investigation, animals were killed by decapitation under anesthesia by i.p. injection of pentobarbital. Kidney cortex tissues were dissected quickly on the ice and stored in liquid nitrogen for reverse transcription-polymerase chain reaction (RT-PCR) and Western blot analysis, respectively.

Determination of uric acid and creatinine levels

Uric acid levels in serum and urine were determined by the phosphotungstic acid method (43). Creatinine levels in serum and urine were measured using standard diagnostic kit purchased.

Western blot analysis

mGLUT9 is expressed in the apical and basolateral membrane of distal convoluted tubules (44). Tissue samples of mouse kidney cortex for mGLUT9, mOAT1, mOCT1, mOCT2 and mGAPDH were prepared as described previously (45). Renal brush border membrane samples for Western blot analysis of mURAT1, mOCTN1 and mOCTN2 and mNa^+-K^+-ATPase were prepared by a modified procedure of Hosoyamada et al. (41). The whole procedure was carried out at 4 °C. Protein contents of kidney brush border membrane and cortex supernatant were determined by the Bradford protein assay reagent (Jiancheng Biotech, Nanjing, P. R. China) with bovine serum albumin as standard.

After resolution of 75 mg of protein by 10 % sodium dodecyl sulfate polyacrylamide gel electrophoresis using Power Pac Basic electrophoresis apparatus (Bio-Rad, Hercules, CA), the protein samples were transferred onto polyvinylidene difluoride (PVDF) membrane (0.45 mm pore size, Pall, USA), which were then blocked with 5 % skim milk and subsequently incubated with primary antibody and horseradish peroxidase-conjugated secondary antibody. Primary antibodies included rabbit polyclonal antibodies against mGLUT9 (diluted 1:1000), mURAT1 (1:2000), mOAT1 (2000), mOAT3 (1:2000), mOCT1 (1:2000), mOCT2 (1:2000), mOCTN1 (1:1000), mOCTN2 (1:1000), mGAPDH (1:5000) and mNa^+-K^+-ATPase (1:1000). For mGLUT9, the C-terminal directed antibody recognizes the isoforms of GLUT9a and GLUT9b. In the present study, western analysis of mouse kidneys revealed a single species of about 55 kDa (44). Immunoreactive bands were visualized by incubation with lumiGLO reagent (Cell Signaling, Beverly, MA) and exposing to X-ray film (Kodak, New Haven, CT). HRP-conjugated goat anti-rabbit or mouse IgG were as the secondary antibody. The contents of target proteins were analyzed via densitometry using Molecular Analyst software (Bio-Rad Laboratories, Hercules, CA) and normalized by the respective blotting from mNa^+-K^+-ATPase or mGAPDH.

Semi-quantitative RT-PCR analysis

Total RNA was extracted from mouse kidney cortex using TRIZOL reagent, following the protocol provided by the manufacturer. RT-PCR was carried out as we described previously (45). mRNA fold changes of target genes relative to the endogenous GAPDH control were calculated as suggested by Schmittgen et al. (46). Briefly, the homogenate was mixed with 200 μl chloroform then centrifuged at 12,000 × *g* for 15 min, 4 °C. Aqueous phase (about 0.5 ml upper layer) was precipitated with equal volume of isopropanol and then centrifuged at 12,000 × *g* for 10 min, 4 °C. After washed by 1ml of 75% ethanol, the final RNA total pellet was resuspended in 20 μl of DEPC water. Reverse transcription was performed with 1 μg RNA using M-MLV reverse transcriptase for cDNA synthesis. PCR amplification was carried out using gene-specific PCR primers. The sequences of gene-specific PCR primers, the appropriate annealing temperature and the length of production were showed in Table 1. PCR products were electrophoresed on 1.2 % agarose gels, visualized with the Bio-Rad ChemiDoc XRS Gel Documentation system, and then quantified using Bio-Rad Quantity One 1-D analysis software. Relative quantitation for PCR products was calculated after normalization to the amount of mGAPDH mRNA levels.

STATISTICAL ANALYSIS

All data were expressed as the mean ± standard error of the mean (S.E.M.) and statistical analysis was performed using a one-way analysis of variance (ANOVA) to determine the levels of significance. A value of $P < 0.05$ was considered statistically significant. And the figures were obtained by the Statistical Analysis System (GraphPad Prism 4, GraphPad Software, Inc., San Diego, CA).

RESULTS

Morin decreases serum uric acid levels in hyperuricemic mice

As expected, mice orally treated with 250 mg/kg potassium oxonate for 1 week exhibited a significant elevation of serum uric acid levels in comparison to normal-vehicle group (Figure1), which was significantly attenuated by one-week treatment with 20, 40 and 80 mg/kg of morin. And 40 and 80 mg/kg morin reduced serum uric acid levels of hyperuricemic mice to the normal. Allopurinol at 2.5 and 5 mg/kg (positive control) also significantly lowered serum levels of uric acid in hyperuricemic mice.

Morin enhances urinary urate excretion and improves renal function in hyperuricemic mice

Urinary levels of uric acid and creatinine were significantly reduced in oxonate-induced hyperuricemic mice compared with normal-vehicle group (Figure 2A and B).

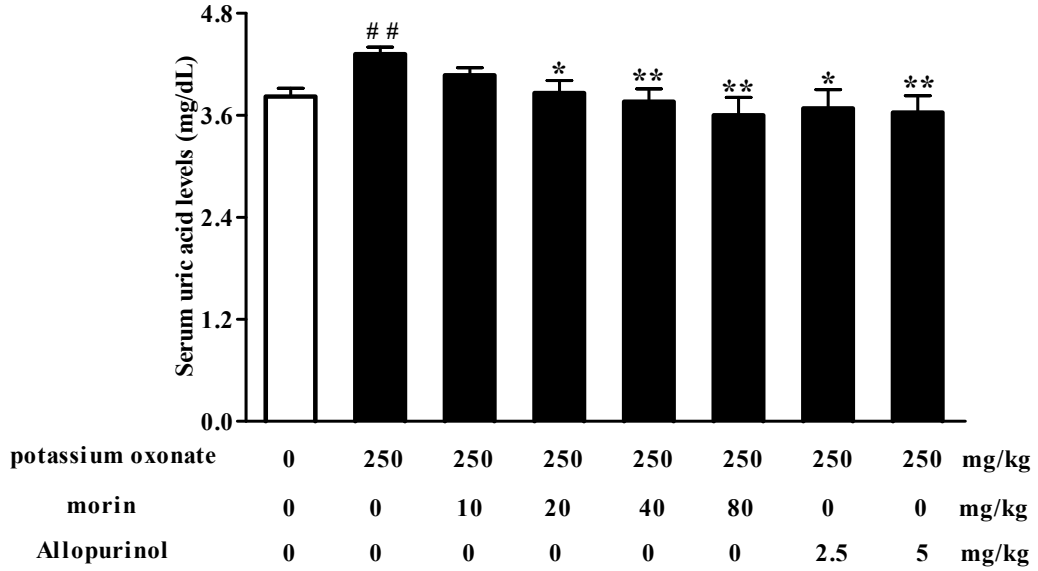


Figure 1. Effects of morin and allopurinol on serum uric acid levels in oxonate-induced hyperuricemic mice. Experiments were performed as described in Methods section. Data were expressed as the mean ± S.E.M. $^{\#\#}$ $P < 0.01$ vs. normal-vehicle animals. $^{*}P < 0.05$ and ** $P < 0.01$ vs. hyperuricemia-vehicle animals.

Hyperuricemic mice also produced a remarkable elevation of serum creatinine levels (Figure 2C) and reduction of Uur/Ucr ratio and FEUA (Figure 2D and E), demonstrating urate underexcretion with the impaired renal function.

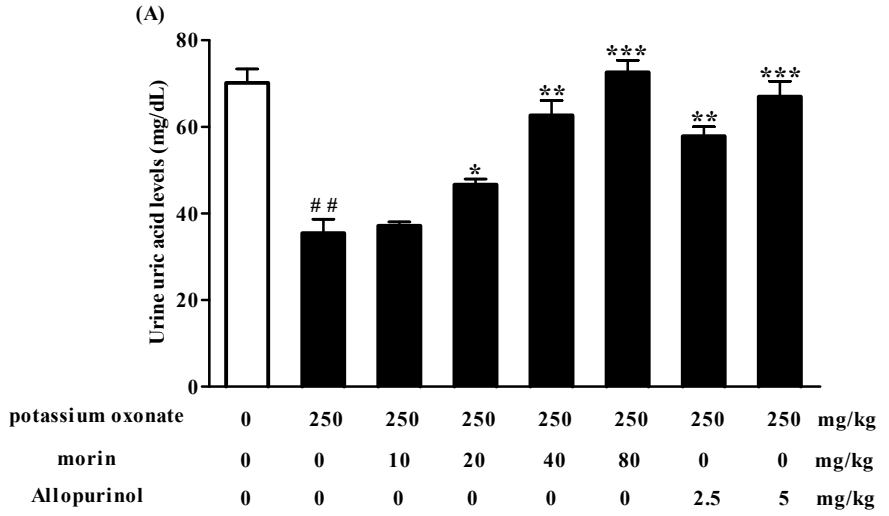
Morin treatment at 20, 40 and 80 mg/kg significantly increased urine uric acid and creatinine levels (Figure 2 A and B), and decreased serum creatinine levels (Figure 2C) in hyperuricemic mice compared with hyperuricemia-vehicle group, resulting in remarkable elevation of Uur/Ucr ratio (40-80 mg/kg) and FEUA (Figure 2D and E). And the highest dose of morin restored alterations of urine and serum creatinine levels and FEUA in hyperuricemic mice to the normal. 2.5 and 5 mg/kg allopurinol significantly restored these alterations in hyperuricemic mice.

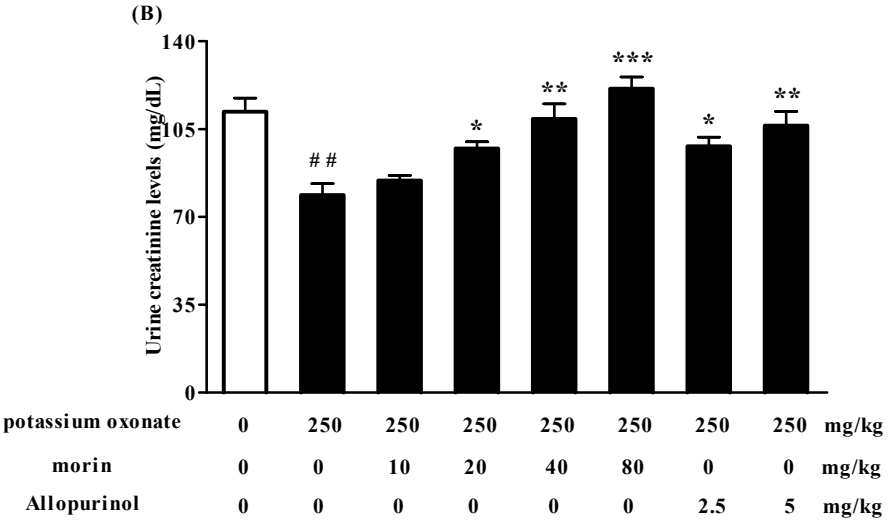
The time-dependent effects of 40 and 80 mg/kg morin as well as 5 mg/kg allopurinol were examined in hyperuricemic mice. Morin at 80 mg/kg on the third day, and at 40 mg/kg on fourth day, decreased serum uric acid and creatinine levels, and increased urinary uric acid and creatinine levels, Uur/Ucr ratio and FEUA in hyperuricemic mice, respectively (Data not shown). And 5 mg/kg allopurinol significantly lowered serum levels of uric acid on the first day (Data not shown). These effects were maintained, indicating that changes in renal excretion of urate mediated by morin or allopurinol might be not affected by renal adaptation during the experimental period.

Morin attenuates oxonate-induced dysregulation of renal mGLUT9, mURAT1 and mOAT1 in mice

Significant increases in renal mGLUT9 and mURAT1 protein levels were observed in oxonate-induced hyperuricemic mice compared with normal-vehicle group (Figure 3A and B), which were likely due to up-regulation of their mRNA levels (Figure 4A and B). In addition, hyperuricemic mice showed significant decreases of renal mOAT1 at both protein (Figure 3C) and mRNA levels (Figure 4C). However, mOAT3 expression was not altered in this model (Figures 3D and 4D).

In comparison to hyperuricemia-vehicle group, protein and mRNA levels of renal mGLUT9 and mURAT1 were significantly suppressed by morin treatment with 20, 40 and 80 mg/kg in hyperuricemic mice (Figures 3A, 3B, 4A and 4B). 5 mg/kg allopurinol reduced mURAT1, but failed to alter mGLUT9 in this model. Moreover, morin treatment remarkably up-regulated renal mOAT1 protein (20, 40 and 80 mg/kg) and mRNA (40 and 80 mg/kg) levels (Figures 3C and 4C). Allopurinol at two doses had similar effect on renal mOAT1 in this model.


(A)
Urine uric acid levels (mg/dL)
80
60
40
20
0
##
*
**
***
**
***
potassium oxonate 0 250 250 250 250 250 250 250 mg/kg
morin 0 0 10 20 40 80 0 0 mg/kg
Allopurinol 0 0 0 0 0 0 2.5 5 mg/kg


(B)
Urine creatinine levels (mg/dL)
140
105
70
35
0
##
*
**
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*
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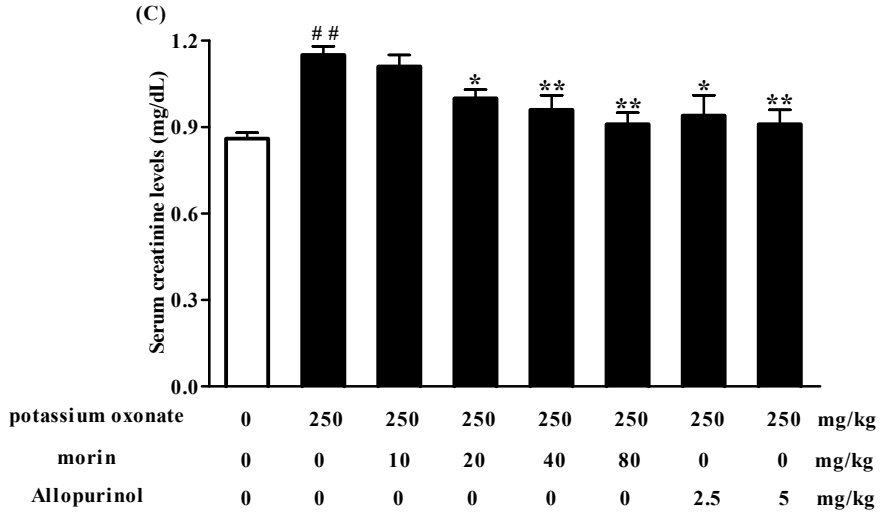
potassium oxonate 0 250 250 250 250 250 250 250 mg/kg
morin 0 0 10 20 40 80 0 0 mg/kg
Allopurinol 0 0 0 0 0 0 2.5 5 mg/kg


(C)
Serum creatinine levels (mg/dL)
1.2
0.9
0.6
0.3
0.0
##
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*
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potassium oxonate 0 250 250 250 250 250 250 250 mg/kg
morin 0 0 10 20 40 80 0 0 mg/kg
Allopurinol 0 0 0 0 0 0 2.5 5 mg/kg

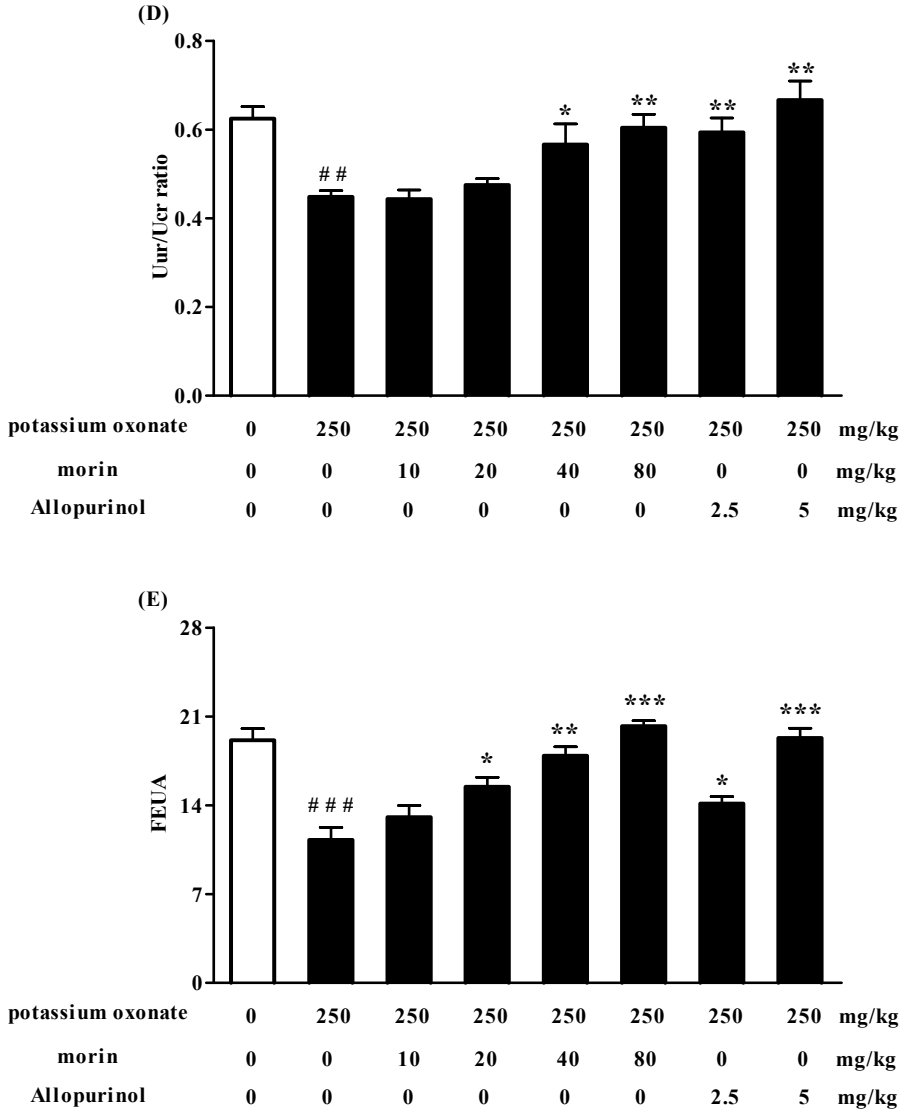
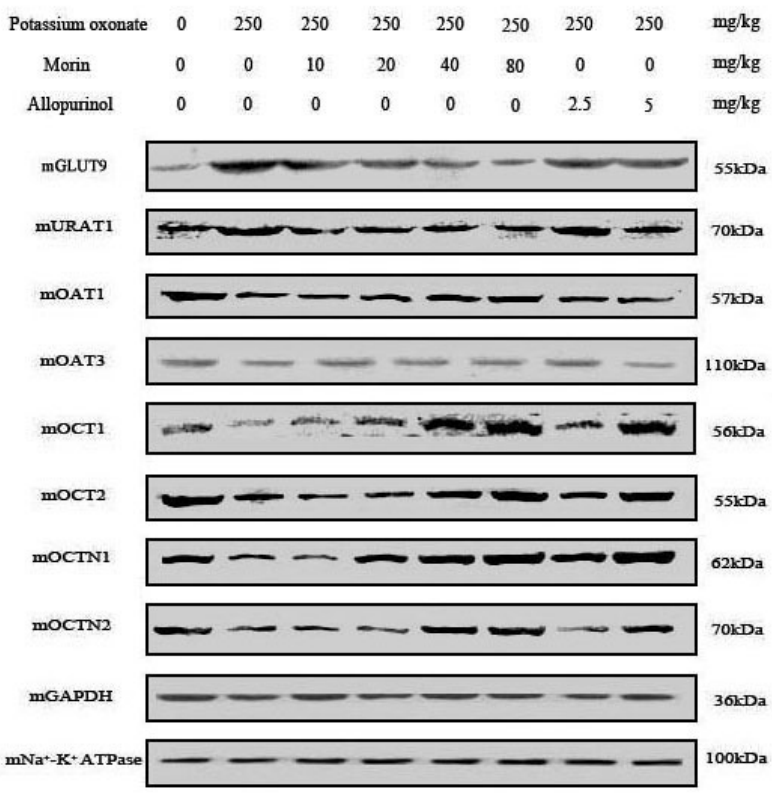


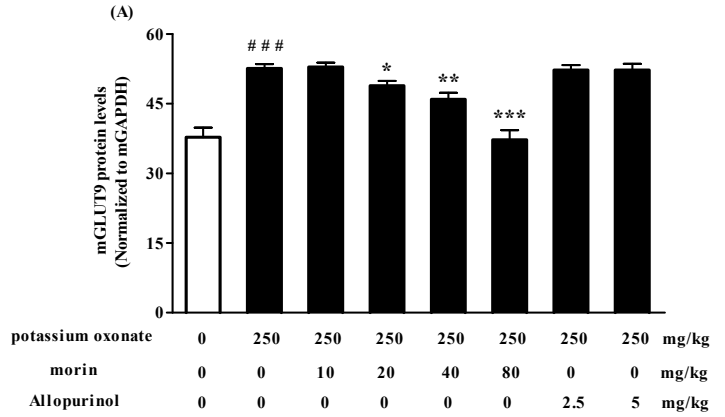
Figure 2. Effects of morin and allopurinol on the levels of urinary uric acid (A) and creatinine (B), serum creatinine (C), as well as urinary uric acid/creatinine ratio (Uur/Ucr) (D) and fractional excretion of uric acid (FEUA) (E) in oxonate-induced hyperuricemic mice. Experiments were performed as described in Methods section. Data were expressed as the mean ± S.E.M. $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ vs. normal-vehicle animals. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. hyperuricemia-vehicle animals.

However, both morin and allpurinol did not change renal mOAT3 in hyperuricemic mice (Figures 3D and 4D).

Morin up-regulates renal mOCT1, mOCT2, mOCTN1 and mOCTN2 in hyperuricemic mice

In comparison to normal-vehicle group, hyperuricemic mice produced down-regulation of renal mOCT1, mOCT2, mOCTN1 and mOCTN2 at both protein (Figure 3E, F, G and H) and mRNA levels (Figure 4E, F, G and H), which were restored by morin treatment at 20, 40 and 80 mg/kg. Allopurinol treatment increased protein and mRNA levels of these transporters except that 2.5 mg/kg allopurinol failed to significantly alter protein levels of renal mOCT1, mOCT2 and mOCTN1 in hyperuricemic mice in the present study.


Potassium oxonate 0 250 250 250 250 250 250 250 mg/kg
Morin 0 0 10 20 40 80 0 0 mg/kg
Allopurinol 0 0 0 0 0 0 2.5 5 mg/kg
mGLUT9 55kDa
mURAT1 70kDa
mOAT1 57kDa
mOAT3 110kDa
mOCT1 56kDa
mOCT2 55kDa
mOCTN1 62kDa
mOCTN2 70kDa
mGAPDH 36kDa
mNa+-K+ ATPase 100kDa


(A)
mGLUT9 protein levels (Normalized to mGAPDH)
60
45
30
15
0
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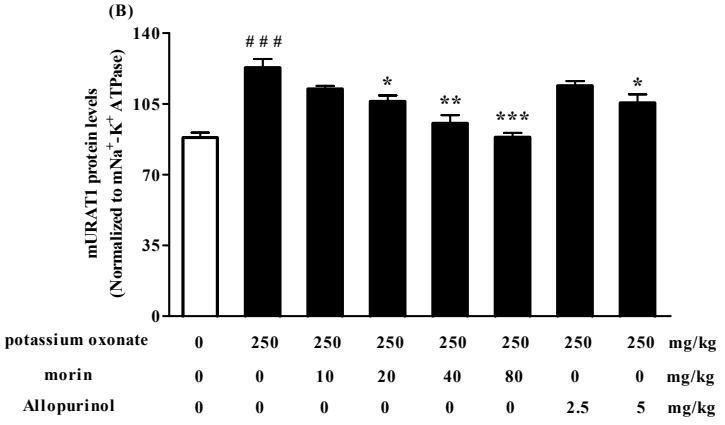
*
**
***
potassium oxonate 0 250 250 250 250 250 250 250 mg/kg
morin 0 0 10 20 40 80 0 0 mg/kg
Allopurinol 0 0 0 0 0 0 2.5 5 mg/kg


(B)
mURAT1 protein levels (Normalized to mNa+-K+ ATPase)
140
105
70
35
0
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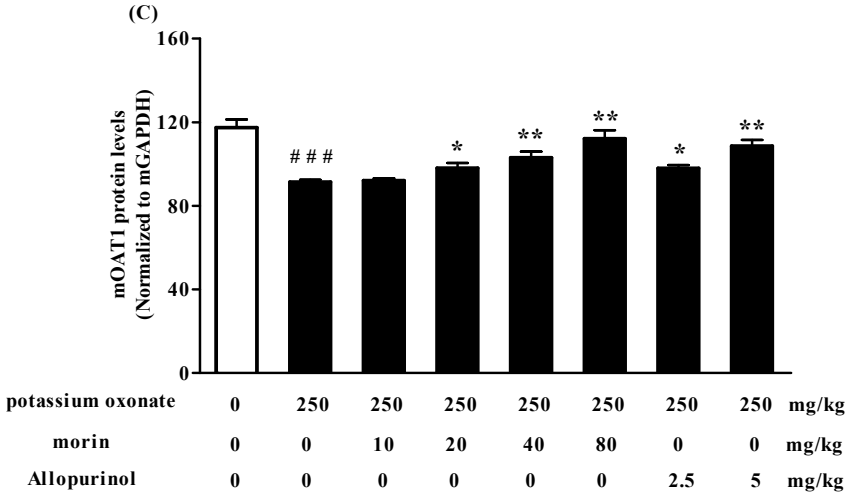
potassium oxonate 0 250 250 250 250 250 250 250 mg/kg
morin 0 0 10 20 40 80 0 0 mg/kg
Allopurinol 0 0 0 0 0 0 2.5 5 mg/kg


(C)
mOAT1 protein levels
(Normalized to mGAPDH)
160
120
80
40
0
# # #
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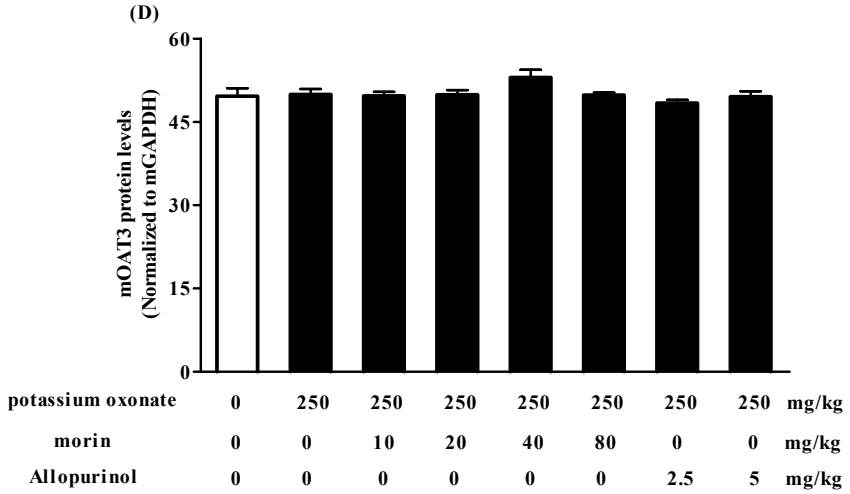
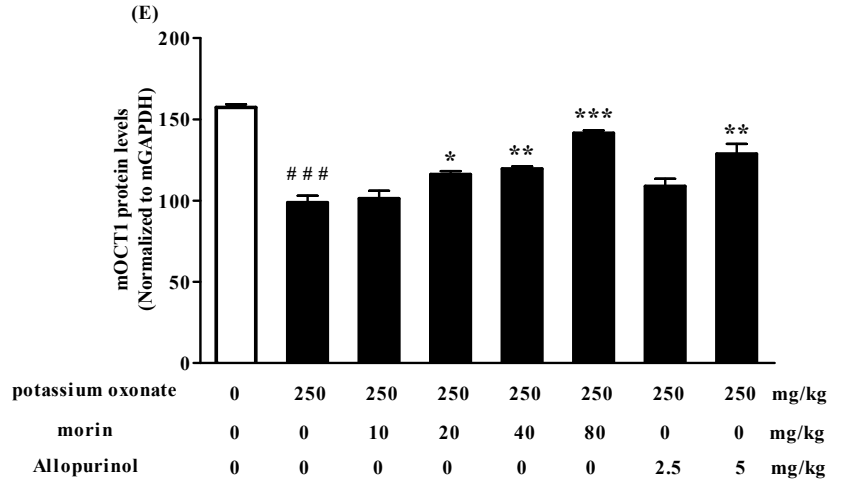
potassium oxonate 0 250 250 250 250 250 250 250 mg/kg
morin 0 0 10 20 40 80 0 0 mg/kg
Allopurinol 0 0 0 0 0 0 2.5 5 mg/kg


(D)
mOAT3 protein levels
(Normalized to mGAPDH)
60
45
30
15
0
potassium oxonate 0 250 250 250 250 250 250 250 mg/kg
morin 0 0 10 20 40 80 0 0 mg/kg
Allopurinol 0 0 0 0 0 0 2.5 5 mg/kg


(E)
mOCT1 protein levels
(Normalized to mGAPDH)
200
150
100
50
0
# # #
*
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potassium oxonate 0 250 250 250 250 250 250 250 mg/kg
morin 0 0 10 20 40 80 0 0 mg/kg
Allopurinol 0 0 0 0 0 0 2.5 5 mg/kg

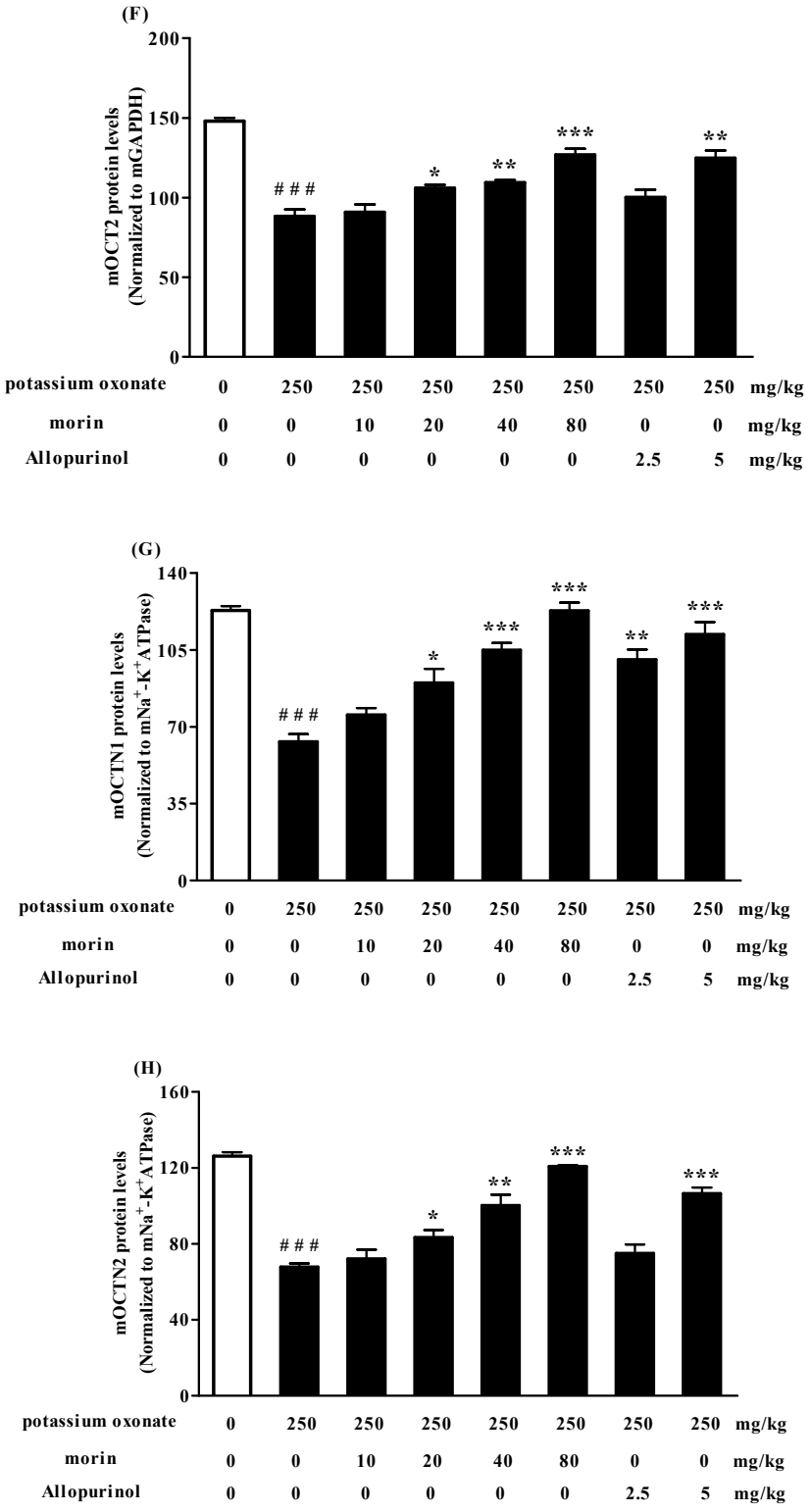
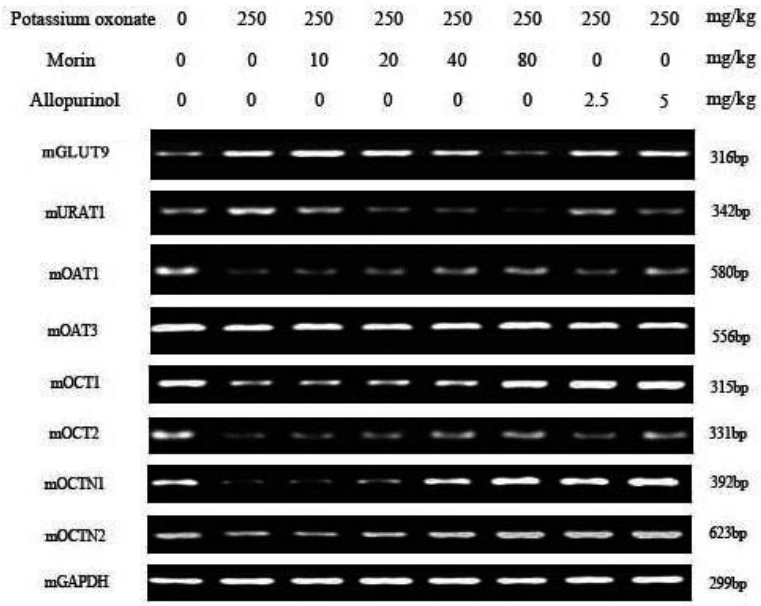
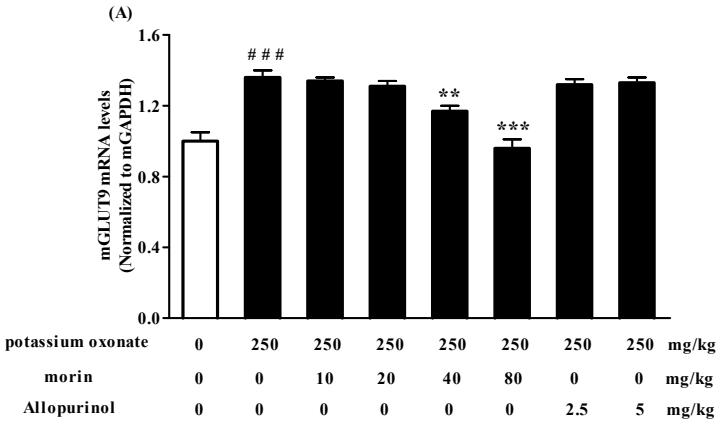
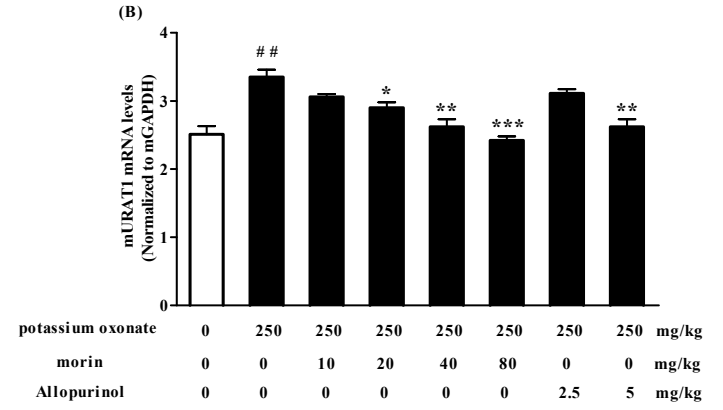
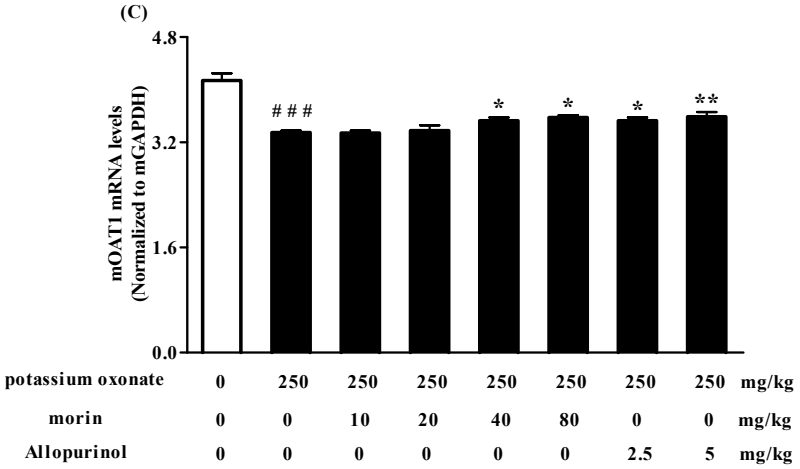


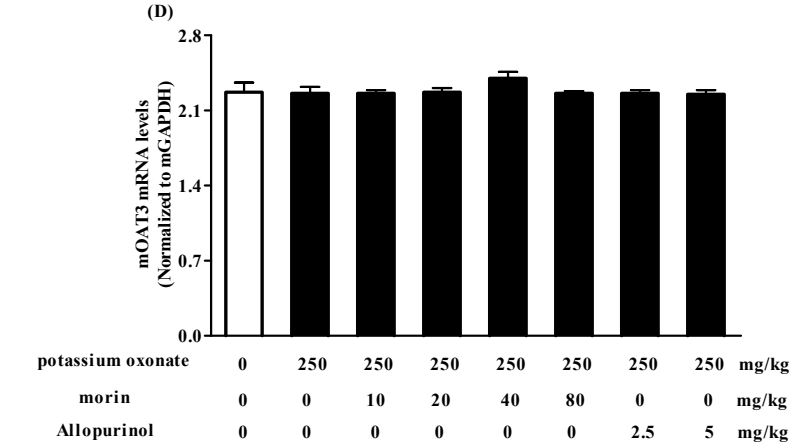
Figure 3. Effects of morin and allopurinol on renal protein levels of mGLUT9 (A), mURAT1 (B), mOAT1 (C), mOAT3 (D), mOCT1 (E), mOCT2 (F), mOCTN1 (G) and mOCTN2 (H) in oxonated-induced hyperuricemic mice. Experiments were performed as described in Methods section. Data were expressed as the mean ± S.E.M. $^{###}$ $P < 0.001$ versus normal-vehicle animals. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. hyperuricemia-vehicle animals.

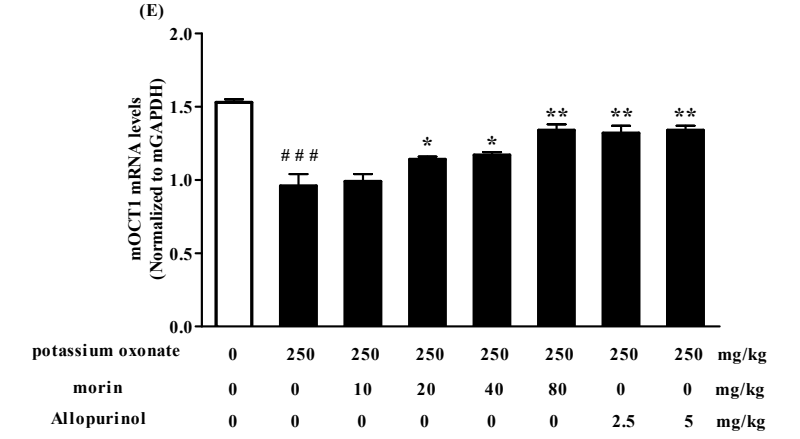

Potassium oxonate 0 250 250 250 250 250 250 250 mg/kg
Morin 0 0 10 20 40 80 0 0 mg/kg
Allopurinol 0 0 0 0 0 0 2.5 5 mg/kg
mGLUT9 316bp
mURAT1 342bp
mOAT1 580bp
mOAT3 556bp
mOCT1 315bp
mOCT2 331bp
mOCTN1 392bp
mOCTN2 623bp
mGAPDH 299bp


(A)
mGLUT9 mRNA levels (Normalized to mGAPDH)
1.6
1.2
0.8
0.4
0.0
###
**
***
potassium oxonate 0 250 250 250 250 250 250 250 mg/kg
morin 0 0 10 20 40 80 0 0 mg/kg
Allopurinol 0 0 0 0 0 0 2.5 5 mg/kg


(B)
mURAT1 mRNA levels (Normalized to mGAPDH)
4
3
2
1
0
##
*
**
***
**
potassium oxonate 0 250 250 250 250 250 250 250 mg/kg
morin 0 0 10 20 40 80 0 0 mg/kg
Allopurinol 0 0 0 0 0 0 2.5 5 mg/kg


(C)
mOAT1 mRNA levels
(Normalized to mGAPDH)
4.8
3.2
1.6
0.0
# # #
*
*
*
**
potassium oxonate 0 250 250 250 250 250 250 250 mg/kg
morin 0 0 10 20 40 80 0 0 mg/kg
Allopurinol 0 0 0 0 0 0 2.5 5 mg/kg


(D)
mOAT3 mRNA levels
(Normalized to mGAPDH)
2.8
2.1
1.4
0.7
0.0
potassium oxonate 0 250 250 250 250 250 250 250 mg/kg
morin 0 0 10 20 40 80 0 0 mg/kg
Allopurinol 0 0 0 0 0 0 2.5 5 mg/kg


(E)
mOCT1 mRNA levels
(Normalized to mGAPDH)
2.0
1.5
1.0
0.5
0.0
# # #
*
*
**
**
**
potassium oxonate 0 250 250 250 250 250 250 250 mg/kg
morin 0 0 10 20 40 80 0 0 mg/kg
Allopurinol 0 0 0 0 0 0 2.5 5 mg/kg

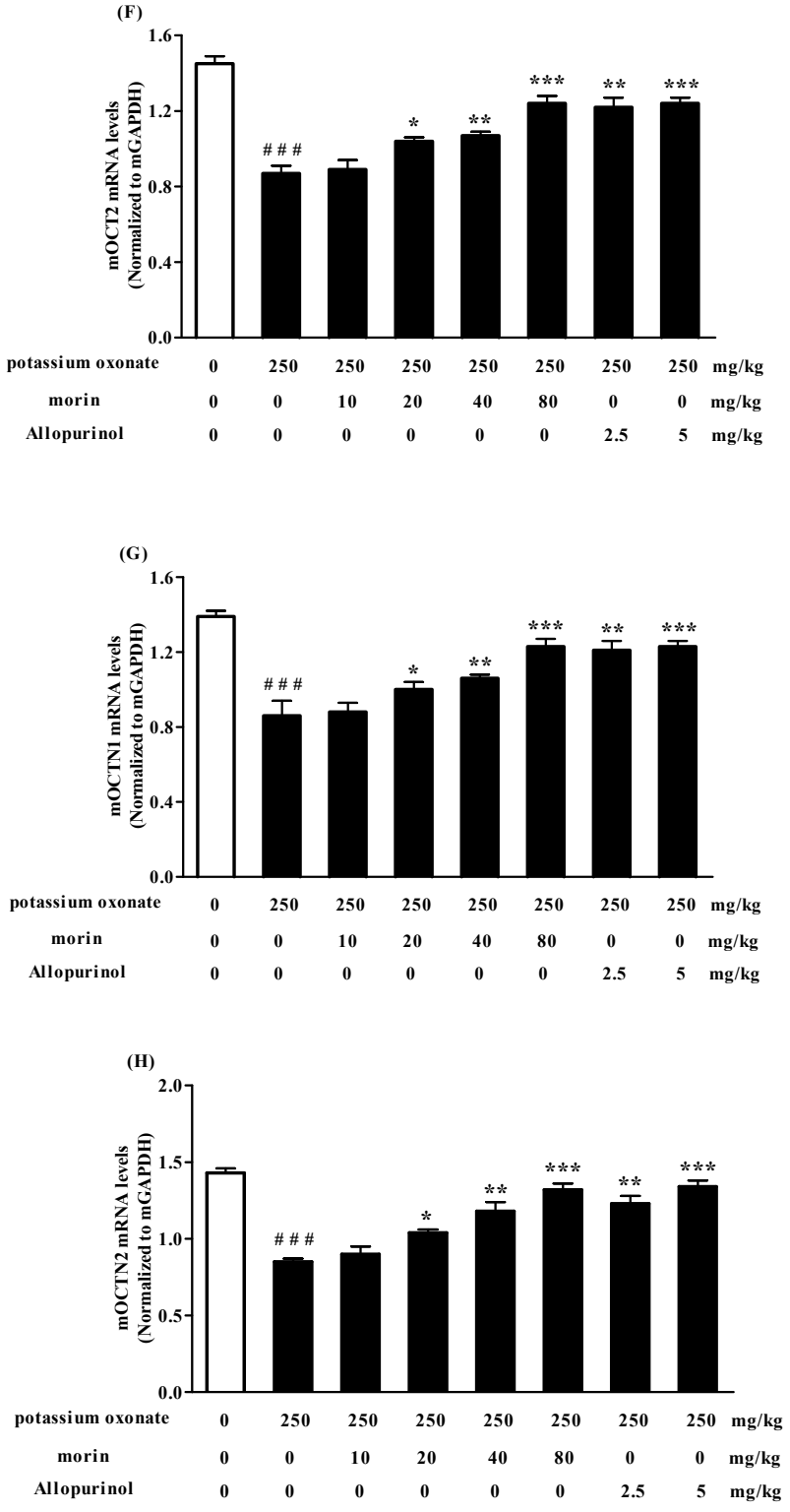


Figure 4. Effects of morin and allopurinol on renal mRNA levels of mGLUT9 (A), mURAT1 (B), mOAT1 (C), mOAT3 (D), mOCT1 (E), mOCT2 (F), mOCTN1 (G) and mOCTN2 (H) in hyperuricemic mice. Experiments were performed as described in Methods section. Data were expressed as the mean ± S.E.M. $^{\#\#}$ $P < 0.01$, $^{\#\#\#}$ $P < 0.001$ vs. normal-vehicle animals. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ vs. hyperuricemia-vehicle animals.

DISCUSSION

The present study confirmed that morin had the uricosuric action and renal function improvement characterized by reduction of serum uric acid levels and elevation of urinary uric acid and creatinine levels, Uur/Ucr ratio and FEUA in potassium oxonate-induced hyperuricemic mice. This and our previous studies showed that oral administration of 250 mg/kg potassium oxonate for seven consecutive days could cause reduction of 24-h urinary uric acid levels on the seventh day in mice (34, 35), which was consistent with other studies in rats with fructose feeding (47) and patients with primary hyperuricaemia and gout (48, 49). Yu et al. (37) report that hyperuricemic rats induced by an i.p. injection of 200 mg/kg potassium oxonate produce the increased urine urate levels than normal group 5 h after the treatment. This discrepancy might be related to differences in approaches for administrating route and collecting urine at specific times. In addition, some of patients with gout exhibit higher urine urate level than controls (42). Unlike most mammals, uric acid is the final product of purine metabolism in human because of the mutational loss of uricase that degrades uric acid to allantoin (50). The breakdown of dietary and cellular purines may induce overproduction of uric acid in the body, resulting in serum urate elevation, possibly accompanying with higher urine urate level in patients.

Renal organic ion transporters may contribute to renal urate handling and function. The present study found that morin treatment decreased protein and mRNA levels of mGLUT9 and mURAT1 in a dose-dependent manner, and increased mOAT1 protein and mRNA levels in the kidney of oxonate-induced hyperuricemic mice.

These results indicate that morin elicits its uricosuric effects cooperatively by down-regulation of renal mGLUT9 and mURAT1 to inhibit urate reabsorption and up-regulation of renal mOAT1 to promote urate secretion in hyperuricemic mice. More importantly, these regulative actions were parallel with its enhancement of uric acid excretion and reduction of serum uric acid levels in hyperuricemic mice. Thus, renal mGLUT9, mURAT1 and mOAT1 may be morin's targets to mediate serum urate level in hyperuricemic mice. Furthermore, morin was found to up-regulate mRNA and protein levels of renal mOCT1, mOCT2, mOCTN1 and mOCTN2 in hyperuricemic mice, paralleling with its renal function improvement. Recent studies have demonstrated that morin has nephroprotective action against OAT-mediated nephrotoxicity (39, 40). These observations suggest that regulation of these renal ion transporters is involved in improvement of renal function in hyperuricemic mice treated with morin.

It was worth noting that mRNA levels of renal mGLUT9 and mURAT1 increased in a manner corresponding to elevation of their protein levels in hyperuricemia of mice. Simultaneously, mRNA levels of renal mOAT1, mOCT1, mOCT2, mOCTN1 and mOCTN2 declined in a manner corresponding to reduction of the protein levels in this model, indicating that oxonate-induced hyperuricemia in mice may alter transcription of these renal transporters. In line with our study, mRNA and protein levels of rOAT1 and rOCT2 changed in the same manner in oxonic acid-induced hyperuricemic rats (17). And morin treatment simultaneously restored alterations in mRNA and protein levels of these renal transporters in the same manner in hyperuricemic mice. To gain a better understanding of the molecular mechanisms underlying the uricosuric activity and renal protection of morin, it would be important to test the effects of morin combined with inhibitors of these renal transporters in this animal model.

Renal organic ion transporters in mice exhibit high identity to the corresponded transporters in human (41, 51-55). If indeed the present findings in mice can be extrapolated to human, morin may be a potential therapeutics for the prevention and treatment of hyperuricemia with renal dysfunction. The significant uricosuric action of morin in hyperuricemic mice is encouraging, particularly in view of nontoxic nature of this natural compound (56, 57) and the reported toxicity problems of clinically hypouricemic agents (30-33). Together with the other favorable characteristics of morin being antioxidation (58) and anti-inflammation (59), its clinical trials on human subjects are highly warranted.

CONCLUSION

The present investigation shows that morin has the uricosuric action cooperatively by decreasing renal urate reabsoption via down-regulation of renal mGLUT9 and mURAT1 and by increasing urate secretion via up-regulation of renal mOAT1 in hyperuricemic mice. Moreover, morin may process renal protection via mediating renal mOCT1,

mOCT2, mOCTN1 and mOCTN2 expressions in hyperuricemic mice. These results therefore strongly suggest that morin may be a potential candidate for the prevention and treatment of hyperuricemia with kidney dysfunction.

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ABBREVIATIONS

GLUT9, glucose transporter 9; URAT1, urate transporter 1; OAT, organic anion transporter; OCT, organic cation transporter; OCTN, organic cation/carnitine transporter; FEUA, fractional excretion of uric acid; Sur, serum uric acid concentration; Scr, serum creatinine concentration; Uur, urine uric acid concentration; Ucr, urine creatinine concentration; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT-PCR, reverse transcription-polymerase chain reaction; M-MLV, moloney murine leukemia virus; PVDF, polyvinylidene difluoride.

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