The Dual Actions of Morin as a Hypouricemic Agent: Uricosuric Effect and Xanthine Oxidase Inhibitory Activity

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a) **Running Title: Mechanisms of the hypouricemic action of morin**

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d) **Abbreviations:** BBMV, brush border membrane vesicles; FEur, fractional excretion of urate; URAT1, urate-anion transporter 1.

e) **Recommended section:** Absorption, Distribution, Metabolism, and Excretion

Abstract

Hyperuricemia is associated with a number of pathological conditions such as gout. Lowering of elevated uric acid level in the blood could be achieved by xanthine oxidase inhibitors and inhibitors of renal urate reabsorption. Some natural compounds isolated from herbs used in traditional Chinese medicine have been previously demonstrated to possess xanthine oxidase inhibitory activities. In the present investigation morin (3, 5, 7, 2', 4'- pentahydroxyflavone), which occurs in the twigs of *Morus alba* L. documented in traditional Chinese medicinal literature to treat conditions akin to gout, was demonstrated to exert potent inhibitory action on urate uptake in rat renal brush border membrane vesicles indicating that this compound acts on the kidney to inhibit urate reabsorption. Lineweaver-Burk transformation of the inhibition kinetics data demonstrated that the inhibition of urate uptake was of a competitive type with a Ki value of 17.4 μM. In addition, morin was also demonstrated to be an inhibitor of xanthine oxidase. Lineweaver-Burk analysis of the enzyme kinetics indicated that the mode of inhibition was of a mixed type, with Ki and Kies values being 7.9 μM and 35.1 μM respectively. Using an oxonate-induced hyperuricemic rat model, morin was indeed shown to exhibit an *in vivo* uricosuric action which could explain, in part at least, the observed hypouricemic effect of morin in these rats. The potential application of this compound in the treatment of conditions associated with hyperuricemia was discussed.

Introduction

Gout is a common systemic rheumatic disease that affects at least 1% of adult male in Western countries (Lawrence et al., 1998; Arromdee et al., 2002). Recent epidemiologic data indicated that the incidence of primary gout has increased by two-fold over the past 20 years (Arromdee et al., 2002). This disease is also rapidly rising in China (Li et al., 1997), due probably to recent changes in dietary habits. Hyperuricemia is a hallmark of gout (Rott and Agudelo, 2003). A serum uric acid level of more than 9 mg/dl is associated with a gouty arthritis incidence of 4.9% (Campion et al., 1987). Pathogenic mechanisms of hyperuricemia include uric acid overproduction or underexcretion caused by aberrations in renal uric acid handling (Liote, 2003). In the general population, 80% to 90% of gout patients are underexcreters (Rott and Agudelo, 2003).

Uric acid is the end product of purine metabolism in humans. It is a weak organic acid with a poor pH-dependent solubility in aqueous solutions. Unlike other mammals, humans have a high serum uric acid level due to the lack of uricase which converts uric acid to the more soluble allantoin. Uric acid production is catalyzed by xanthine oxidase in the liver, and ~70% of the daily output of uric acid is excreted through the kidney (Marangella, 2005). A three-component model has been proposed for uric acid excretion in humans. This includes filtration, reabsorption and secretion. Recently, a urate-anion transporter (URAT1) was identified in the brush border membrane of the proximal tubule in human kidney (Enomoto et al., 2002). This transporter is believed to play a significant role in regulating serum uric acid level through reabsorption of urate from the lumen to the cytosol in kidney tubules.

The treatment of gout entails several approaches. Apart from the symptomatic relief of pain and swelling associated with inflammation, drugs which could help to reduce the elevated uric acid level in the blood are employed as therapeutic agents. These include

xanthine oxidase inhibitors in which allopurinol is the most often prescribed. Inhibitors of renal urate reabsorption such as probenecid and benzbromarone are also employed as hypouricemic agents. However, these existing antihyperuricemic agents possess some undesirable effects such as hypersensitivity towards allopurinol (Bomalaski and Clark, 2004) and hepatotoxicity associated with benzbromarone (Schlesinger, 2004). Thus, search for alternative antihyperuricemic agents with a more favorable toxicological profile is highly warranted. In China, natural herbs have been used for a long time in the treatment of gout and hyperuricemia-related disorders (Chou and Kuo, 1995). We have previously reported the identification of some natural compounds from these herbs possessing xanthine oxidase inhibitory activities (Kong et al., 1999; Kong et al., 2000). We have also reported that some natural compounds might exert their hypouricemic action via mechanisms other than xanthine oxidase inhibition (Kong et al., 2002). In the present investigation we report that morin (3, 5, 7, 2', 4'- pentahydroxyflavone, Fig. 1), which occurs in a herb used to treat gouty conditions in traditional Chinese medicine, is a potent inhibitor of urate reabsorption in the kidney, in addition to its inhibitory effects on xanthine oxidase. The dual actions of this compound provide an explanation for its *in vivo* hypouricemic effect.

Materials and Methods

Materials. Unless otherwise stated, all chemical reagents and compounds were obtained from Sigma Chemical Co. [8-^{14}C]Urate (55 mCi/mmol) was purchased from American Radiolabeled Chemicals Inc. Mixed cellulose membrane filters (diameter = 25 mm, pore size = 0.45 μm) and 30,000 M.W. cut off centrifugal filters (Catalog No. UFC3LTK00) were purchased from Millipore Co. HI-SAFE II scintillant was supplied by Perkin Elmer Inc. The Creatinine Kit (Catalog No. 0420-250) was purchased form Stanbio Laboratory. Metabolic cages and experimental animals were provided by the Laboratory Animal Services Center of The Chinese University of Hong Kong.

***In vitro* action of morin on urate uptake in rat renal brush border membrane vesicles (BBMV).** Preparation of BBMV: BBMV were prepared from rat kidney cortex by the magnesium aggregation method (Dan and Koga, 1990). Briefly, adult male Sprague-Dawley rats (300g body weight) were first anesthetized by intraperitoneal injection of pentobarbital. The kidneys were removed rapidly and the renal cortices were cut out. All subsequent steps were performed at 4°C. The cortices were homogenized in 250 mM mannitol in 10 mM Tris-HEPES (pH 7.5) using a tissue tearor (Biospec, model 985370, 6 x 20 s at speed 5). The homogenate was centrifuged at 2,400 × g for 10 min and the supernatant obtained was centrifuged again at 28,000 × g for 20 min. After discarding the supernatant, the loosely packed brush border membrane layer was gently washed off the bottom densely packed brown pellet and resuspended in the homogenizing buffer using a Dounce homogenizer. $MgSO_4$ was then added to a final concentration of 10 mM. The suspension was allowed to stand for 20 min and then centrifuged at 2,400 × g for 20 min. The supernatant obtained was carefully transferred to a new tube and re-centrifuged twice

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at 2,400 × g for 20 min. The final supernatant was centrifuged again at 28,000 × g for 20 min. The pellet obtained was suspended in a small volume of 50 mM potassium phosphate buffer (pH 7.5) containing 150 mM mannitol and 2 mM $MgSO_4$ at a final protein concentration of 15-20 mg/ml. The purity of BBMV was assessed by the fold enrichment of the brush border membrane marker enzyme alkaline phosphatase, which was quantitated by a spectrophotometric method using *p*-nitrophenylphosphate as the substrate (Winchester et al., 1999). Protein concentration was measured by the method of Bradford (1976), using bovine serum albumin as the standard.

Urate uptake into BBMV: [^{14}C]Urate uptake into BBMV was determined at room temperature by the rapid filtration method (Edwards et al., 1996) in the presence of an OH^- gradient. Urate uptake was initiated by adding 10 µl of the freshly prepared BBMV (in the pH 7.5 buffer) into 90 µl of uptake buffer (150 mM mannitol, 2 mM $MgSO_4$, 50 mM potassium phosphate, pH 6.0), containing 38 µM [^{14}C]urate with or without the putative inhibitor. After exactly 12 s, 4 ml of pre-chilled uptake buffer were added to stop the uptake process. The sample was immediately filtered through a 0.45 µm mixed cellulose membrane kept under suction. The BBMV held on the filter were washed twice with the uptake buffer. The radioactivity on the filter was counted on a liquid scintillation counter (Beckman LS2900TR) after adding 4 ml of the scintillant. In most experiments, urate uptake was also measured in the absence of an OH^- gradient in which the pH value of the uptake buffer was 7.5 instead of 6.0. Urate uptake through the urate-anion transporter was calculated by subtracting the uptake value in the absence of an outwardly directed OH^- gradient ($pH_{in} = pH_{out} = 7.5$) from that in the presence of the gradient ($pH_{in} = 7.5$ and $pH_{out} = 6.0$). The amount of [^{14}C]urate bound on the filter in the absence of BBMV was also measured to serve as the blank for the uptake assessment. The putative uptake inhibitor was originally dissolved in dimethylsulfoxide and diluted by the appropriate uptake buffer

before the assay. The final concentration of dimethylsulfoxide in the assay was 1%. This concentration of dimethylsulfoxide was determined in initial experiments to exert minimal effects on the uptake assay. Probenecid was used as a positive control for the uptake inhibition.

***In vitro* inhibitory activity of morin on xanthine oxidase.** The activity of xanthine oxidase was measured spectrophotometrically by following the increase in absorbance at 295 nm (Kong et al., 2000). The enzyme assay was initiated by the addition of 100 µl of xanthine (1.2mM) to 900 µl of assay buffer containing 0.1 M sodium pyrophosphate (pH 7.5), 0.08 U/ml xanthine oxidase, with or without the putative inhibitor. The putative inhibitor was initially dissolved in dimethylsulfoxide and diluted with the assay buffer. The final concentration of dimethylsulfoxide in the assay was 5%. This concentration of dimethylsulfoxide was shown in initial experiments to exert no appreciable effect on the enzyme activity. In the enzyme kinetics study, the xanthine oxidase activity was determined at 2, 3, 4, 6, 7, 9, 12, 60 and 120 µM xanthine. The xanthine oxidase concentration used was 0.01 U/ml to allow a more accurate determination of enzyme activity at low substrate concentrations.

***In vivo* action of morin on plasma and urine urate levels in oxonate-induced hyperuricemic rats.** Treatment of animals: A hyperuricemic rat model was adopted by the intraperitoneal injection of oxonate (Yonetani and Iwaki, 1983; Yamada et al., 1999; Kong et al., 2002). The *in vivo* actions of morin were studied by housing the rats in metabolic cages (Dan et al., 1994). Rats were divided into 5 groups: the normal group receiving vehicle only; the hyperuricemic control group receiving potassium oxonate only; and three morin groups each receiving potassium oxonate and in addition different

amounts (5, 20, 80 mg/kg) of morin. All rats (200 g body weight each) were housed individually in metabolic cages in an air-conditioned room (25 °C), and had free access to food and water during the period of the experiment. After stabilized in the experimental set up for one day, rats were orally administered with the vehicle or the designated amount of morin, followed immediately by an intraperitoneal injection of vehicle/potassium oxonate (200 mg/kg). Morin and oxonate were administered via two different routes to avoid any possible interaction between the two agents. Morin was prepared in 0.3% Tween 20 while oxonate was suspended in 0.8% sodium carboxymethylcellulose. Before the injection, the suspensions were sonicated at 4 °C for 20 min and vortexed vigorously. After the treatments, urine was collected for 5 h. At the end of the 5 h period, rats were anesthetized by intraperitoneal injection of pentobarbital and blood was sampled by cardiopuncture. Blood was centrifuged at 3,000 × g for 10 min at 4 °C for plasma separation. The plasma and urine samples were stored at –20 °C before analyses of uric acid and creatinine concentrations.

Determination of uric acid and creatinine contents: Uric acid level was determined by an HPLC method using a reverse phase C18 column (Kock et al., 1995) on a Beckman Coulter Gold HPLC system with a manual injector. The plasma and urine samples were pretreated by filtering through 30,000 M.W. cut off centrifugal filters. After suitable dilution with HPLC-grade water, 20 µl of the sample was injected into the column and eluted with the mobile phase containing 27.5 mM NaH_2PO_4 (pH 4.2), 2.5 mM octanesulfonic acid and 10% acetonitrile at a flow rate of 1 ml/min. The eluate was monitored for absorbance at 292 nm. On the other hand, the plasma and urine creatinine levels were determined spectrophotometrically using the Stanbio Creatinine Kit. The fractional excretion of urate (FEur) was then calculated as follows to assess the uricosuric effect of the test compound (Dan et al., 1994):

FEur = ([urine urate]/[plasma urate])/([urine creatinine]/[plasma creatinine]) -- Equation 1

Statistical analysis. Values are expressed as mean values ± S.E.M. IC_{50} values were determined from the concentration dependence curves. Data from the *in vivo* experiments were analyzed using one-way ANOVA followed by the Dunnett's multiple comparison test to determine the level of significance. *P* values of <0.05 were taken as statistically significant.

Results

The BBMV fraction that we obtained exhibited a 12.9±0.4 fold increase in the specific activity of alkaline phosphatase when compared with the homogenate, indicating that it was highly enriched in the brush border membrane component, in line with published results in the literature (Dan and Koga, 1990; Edwards et al., 1996; Yamada et al., 2000). In the presence of the pH gradient (7.5/6.0), urate uptake proceeded rapidly in a linear manner within 20 s and then gradually leveled off (Fig. 2), again consistent with reports in the literature (Dan and Koga, 1990; Edwards et al., 1996; Yamada et al., 2000), indicating the robustness of our system. Thus in subsequent experiments urate uptake was determined at 12 s in order to obtain the initial velocity. In the absence of the pH gradient, urate uptake was about 50% of that obtained in the presence of the gradient.

The inhibitory effect of morin on the OH^--dependent urate uptake was demonstrated using the validated BBMV system, using probenecid as the control inhibitor. At a concentration of 25 μM, morin inhibited OH^--dependent urate uptake by 58.8% while at the same concentration probenecid had no inhibitory activity at all (Fig. 3). On the other hand, morin did not show any significant effect on urate uptake in the absence of the pH gradient (data not shown). Fig. 3 shows the dose-dependent inhibition of morin on the OH^--dependent urate uptake. IC_{50} was observed at a concentration of 18.0±2.5 μM. Subsequent Lineweaver-Burk transformation of the uptake kinetics data revealed that morin inhibited the OH^--dependent urate uptake in a competitive manner (Fig. 4), with a Ki value of 17.4±3.5 μM.

Morin also inhibited the activity of xanthine oxidase in a concentration dependent manner with an IC_{50} value of 44 μM (Fig. 5). The inhibition of morin on xanthine oxidase was shown to be reversible as the enzyme activity could be recovered after removal of the inhibitor by dilution. Lineweaver-Burk analysis of the enzyme kinetics data showed that

the inhibition of xanthine oxidase by morin was of a mixed type. In mixed inhibition, the inhibitor can bind to the free enzyme as well as to the enzyme-substrate complex (Palmer, 1995). Thus, two inhibitor constants can be defined, with Ki (inhibitor constant on the enzyme) and Kies (inhibitor constant on the enzyme-substrate complex) values determined to be 7.9 μM and 35.1 μM, respectively (Fig. 6).

Figure 7 summarizes the *in vivo* actions of morin on the oxonate-induced hyperuricemic rats. Oxonate treatment resulted in 57.5% increase in the plasma uric acid level 5 h after treatment while co-administration of morin could effectively reduce such increase in a dose-dependent manner (Fig. 7A). At a morin dose of 80 mg/kg of body weight, the plasma uric acid level returned to that of the normal rats. Similarly, the urine urate level was significantly increased (by 3.2-fold) in the oxonate-induced hyperuricemic control rats. Co-administration of morin (5 and 20 mg/kg) further increased the urine urate level. However, at a high morin dose of 80 mg/kg, a decrease in the urine urate level was recorded (Fig. 7B). On the other hand, all the other parameters measured including urine and plasma creatinine levels and urine volume remained essentially the same for the five groups of rats (Figs. 7C, 7D, 7E). Thus, morin, at a concentration of 20 mg/kg, could significantly increase (by 2-fold) the fractional excretion of urate in the hyperuricemic rats (Fig. 8).

Discussion

Anti-hyperuricemic agents are commonly employed for the treatment of chronic gouty arthritis (Rott and Agudelo, 2003). Current anti-hyperuricemic agents in use include uricosuric agents, xanthine oxidase inhibitors and the enzyme urate oxidase (Schlesinger, 2004). Uricosuric agents such as probenecid, sulfinpyrazone and benzbromarone act on the urate anion transport pathway and inhibit renal proximal tubular urate reabsorption. Xanthine oxidase inhibitors such as allopurinol interfere with the conversion of hypoxanthine to xanthine and then to uric acid. Urate oxidase oxidizes urate to the more soluble allantoin which is more readily excreted through the kidney.

Although a number of anti-hyperuricemic agents are available, their utilization is sometimes limited by the associated undesirable side effects. In general, allopurinol is the drug of choice. However, about 5% of patients are unable to tolerate its adverse side effects which include gastrointestinal irritation, bone marrow suppression and hypersensitivity syndromes ranging from simple skin rash to life-threatening conditions in which the patients develop toxic epidermal necrolysis, fever, hepatitis, eosinophilia and worsening renal function (Yale et al., 1996; Umpiérrez et al., 1998; Bomalaski and Clark, 2004). Uricosuric agents are used in patients with allopurinol-allergic syndromes as well as in underexcreters with normal renal function and no history of urolithiasis. These uricosuric agents are also not without problems. For example, benzbromarone can cause fulminant hepatotoxicity and this in fact had led to its withdrawal from the French market in 2001 (Bieber and Terkeltaub, 2004), while probenecid and sulfinpyrazone have been reported to be nephrotoxic when used to treat hyperuricemia associated with moderate chronic renal insufficiency (Terkeltaub, 2003). Enzyme therapy using urate oxidase is not widely used and is still undergoing clinical trials in the U.S. Thus the search for better anti-hyperuricemic agents is highly warranted. The present study represents such an effort.

From our studies on natural compounds, morin emerged as a potent urate-lowering agent possessing dual mechanisms of action.

In the general population, 80% to 90% of gout patients are underexcreters (Rott and Agudelo, 2003), indicating the importance of the kidney as a therapeutic target. Renal handling of urate is complicated. Not only does urate transport vary among species, but there also exists a bi-directional transport across renal tubule cells. Normally, less than 5% of circulating urate is bound to plasma proteins and thus most of the urate can be freely filtered through the renal glomerulus (Rafey et al., 2003). More than 90% of the filtered urate is reabsorbed in mature humans (Maesaka and Fishbane, 1998). Recently, Enomoto et al. (2002) identified the long-hypothesized urate transporter (URAT1) in human kidney. Genetic defects of URAT1 cause idiopathic renal hypouricemia (Enomoto et al., 2002). Therefore, it is believed that this urate-anion exchanger is involved in the regulation of blood urate level by mediating urate reabsorption. Immunohistochemical analysis revealed the location of URAT1 protein in the epithelial brush border membrane of the proximal tubules in the human renal cortex (Enomoto et al., 2002). In rats, urate excretion is similar to that in humans. Urate excretion in both species undergoes bi-directional tubular transport with the final result being net reabsorption (Edwards et al., 1996). The rat renal BBMV is therefore a useful system for studying potential uricosuric agents.

The purity of our BBMV preparation was assessed by the enrichment in alkaline phosphatase activity. The specific enzyme activity enrichment obtained was 12.9±0.4 fold, similar to that reported in the literature (Dan and Koga, 1990; Edwards et al., 1996). Morin could significantly inhibit the OH^--dependent urate uptake in BBMV. Its inhibition was much stronger than that of probenecid. The IC_{50} values for morin was 18 μM, while that for probenecid was 200 μM which was similar to the previously reported value of 174 μM (Dan and Koga, 1990).

The *in vivo* experiments demonstrated that morin could indeed exert hypouricemic and uricosuric actions in the oxonate-induced hyperuricemic rats. We have used a dose of 200 mg oxonate per kg body weight, since a higher dose of the drug would result in a much higher increase in urine urate level overshadowing the uricosuric effects being investigated (Sugino and Shimada, 1995). Potassium oxonate is an inhibitor of uricase. An intraperitoneal injection of oxonate could partially block the conversion of uric acid to allantoin and hence artificially elevate the plasma uric acid level in rats to provide a hyperuricemic animal model (Johnson et al., 1969; Osada et al., 1993). Morin, at a concentration of 80 mg/kg, restored the plasma uric acid level of the hyperuricemic rats to that of the normal group. However, at this concentration of morin, the decrease in plasma uric acid level could not be explained simply by its uricosuric action because the urate excreted was actually less for the morin-treated hyperuricemic rats when compared with the hyperuricemic control rats (Fig. 7B). Instead, the hypouricemic action of morin at 80 mg/kg was probably due to the inhibitory effect of morin on xanthine oxidase. The inhibition of morin on xanthine oxidase (IC_{50} = 44 μM) is moderate when compared with allopurinol (IC_{50} = 5.43 μM)(Fernandes et al., 2002). At a high dose of morin, however, xanthine oxidase would be significantly inhibited. Thus, although further metabolism of uric acid was inhibited by oxonate, the plasma uric acid level was still low because its synthesis was inhibited. This was probably the reason for the observed low level of urate excretion at this high dose of morin.

Morin is a natural compound found in fig and other moraceae (Kang et al., 2004). Incidentally, this compound occurs abundantly in the twigs of *Morus alba* L. which is documented in traditional Chinese medicine as a herb used in the treatment of conditions akin to gouty arthritis in modern medicine. Our results therefore provide a scientific basis for the clinical application of this herb. In addition, the favorable safety profile of this

natural compound (Wu et al., 1994) makes it a potential candidate worthy of further investigations.

Elevated uric acid levels have been associated with a number of pathological conditions in humans. On the other hand, uric acid also possesses antioxidant activities. The loss of the uricase gene and hence a high level of plasma urate in humans has been speculated to be an evolutionary mechanism that allows a longer lifespan. In this regard, it is interesting to point out that morin also possesses antioxidant activities (Wu et al., 1994; Wu et al., 1995; Kok et al., 2000). This antioxidant property of morin is actually an added advantage for this compound as a hypouricemic agent because the attenuated antioxidant capacity due to the lowering of uric acid level could be compensated by this natural compound. Further studies to delineate its clinical efficacy and to determine the appropriate dosage are highly warranted.

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Footnotes

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Legends for Figures

Figure 1 Structure of morin.

Figure 2 Time course of urate uptake into rat renal BBMV in the presence of an outwardly directed OH^- gradient (pH_{in} = 7.5 and pH_{out} = 6.0). Each datum point is the average value from 3 determinations.

Figure 3 Concentration-dependent inhibition of OH^--dependent urate uptake in rat renal BBMV by morin and probenecid. OH^--dependent urate uptake was calculated as the difference between uptake in the presence of an outwardly directed OH^- gradient (pH_{in} = 7.5 and pH_{out} = 6.0) and uptake in the absence of the gradient (pH_{in} = pH_{out} = 7.5). The inhibitory activities of morin and probenecid were represented as the percentage of the control where no inhibitor was added. For morin, the data are the mean values ± S.E.M. of 4 independent experiments each performed in triplicates while the data for probenecid are the mean values of 4 determinations. The 100% value of urate uptake in the control corresponds to 20.8 pmol/mg protein for the morin part and is 22.0 pmol/mg protein for the probenecid part.

Figure 4 A Lineweaver-Burk plot of the inhibition of urate uptake by morin. OH^--dependent urate uptake was measured at various concentrations of urate, viz. 76 µM, 38 µM, 19 µM, 9.5 µM and 4.75 µM, in the absence (●) and presence (▲) of 25 µM morin. Three independent experiments were performed and the results of a representative one is shown. The inhibition constant of morin was determined to be 17.4 ± 3.5 µM.

Figure 5 Concentration-dependent inhibition of xanthine oxidase activity by morin. The activity of xanthine oxidase was measured spectrophotometrically by following the increase in absorbance at 295 nm in an assay medium containing 120 μM xanthine, 0.08 U/ml xanthine oxidase and different concentrations of morin. Data presented are the mean values ± S.E.M. of 4 independent experiments.

Figure 6 Inhibition kinetics of xanthine oxidase by morin. Panel A shows the Lineweaver-Burk plots in the absence of morin (■) or at different concentrations of morin: 5 μM (▲), 15 μM (●), and 30 μM (◆). Secondary plots to calculate the inhibition constants are shown in Panel B (Ki) and Panel C (Kies). The assay medium contained 0.01 U/ml xanthine oxidase, different concentrations of xanthine and the indicated concentrations of morin. Each datum point is the average value from two determinations in a representative experiment.

Figure 7 *In vivo* actions of morin on oxonate-treated rats. Experiments were performed as described in Materials and Methods. Urine was collected for 5 h. Blood sample was obtained by cardiopuncture at the end of the 5 h period. The samples were analyzed for plasma urate level (Panel A), urine urate level (Panel B), urine creatinine level (Panel C), plasma creatinine level (Panel D), and urine volume (Panel E). N stands for the normal rats without drug treatment. C stands for the hyperuricemic control rats with oxonate treatment. The dosages of morin used are indicated as shown. Data are expressed as the mean values ± S.E.M. for 5 rats (*P <0.05, *** P<0.001 versus the hyperuricemic control animals).

Figure 8 *In vivo* action of morin on the fractional excretion of urate in oxonate treated rats. The notations are the same as in Figure 7. The data shown are the mean values ± S.E.M. of 5 rats (***P <0.001 versus the hyperuricemic control animals).

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Figure 1

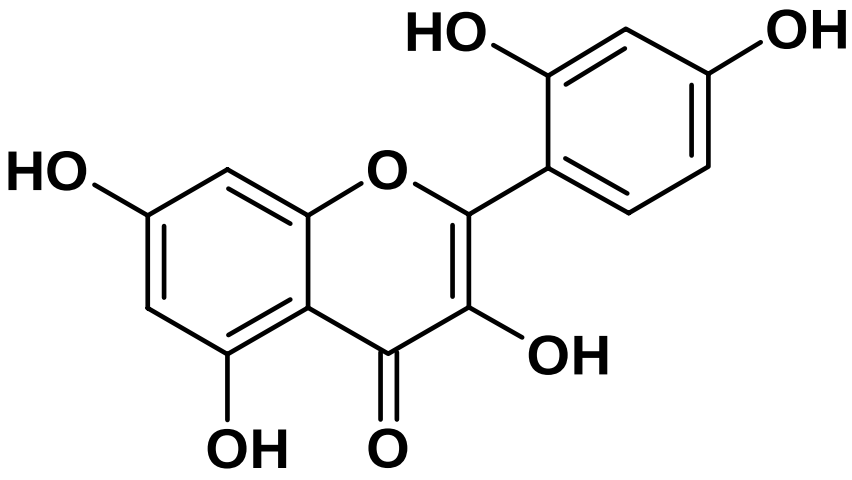

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Figure 2

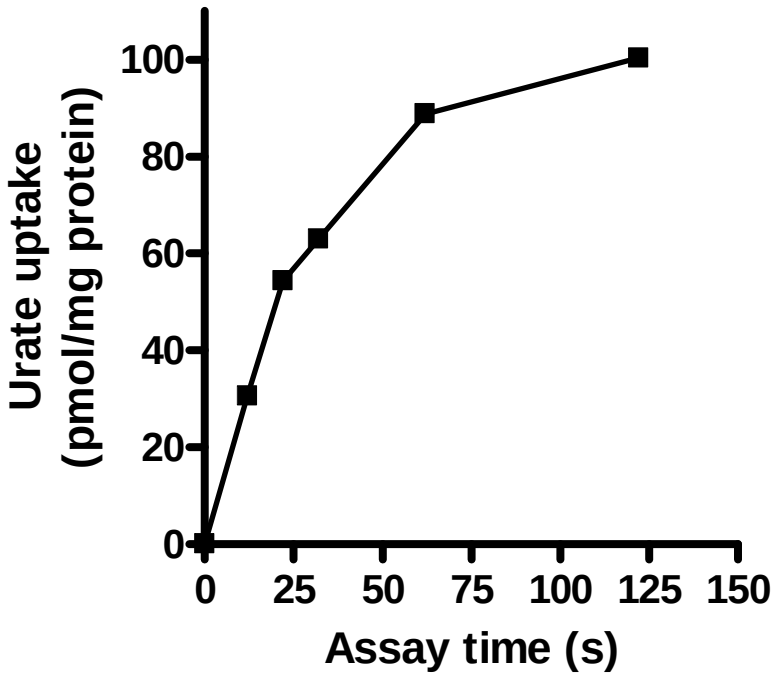

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Figure 3

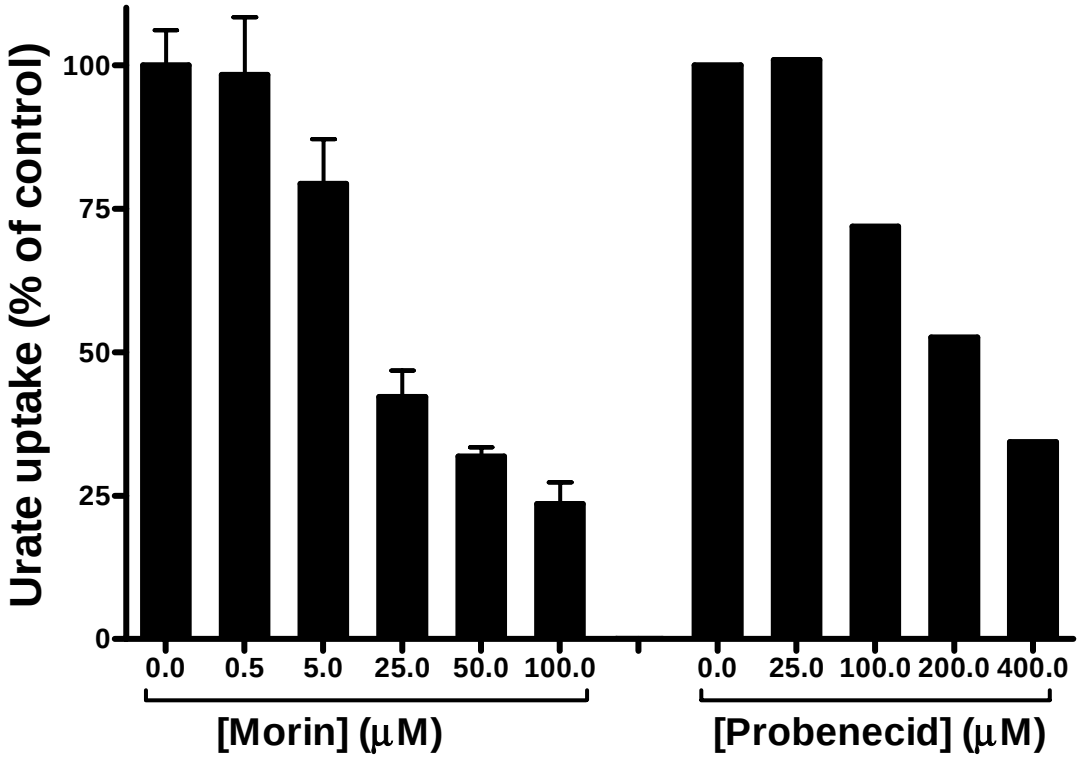

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Figure 4

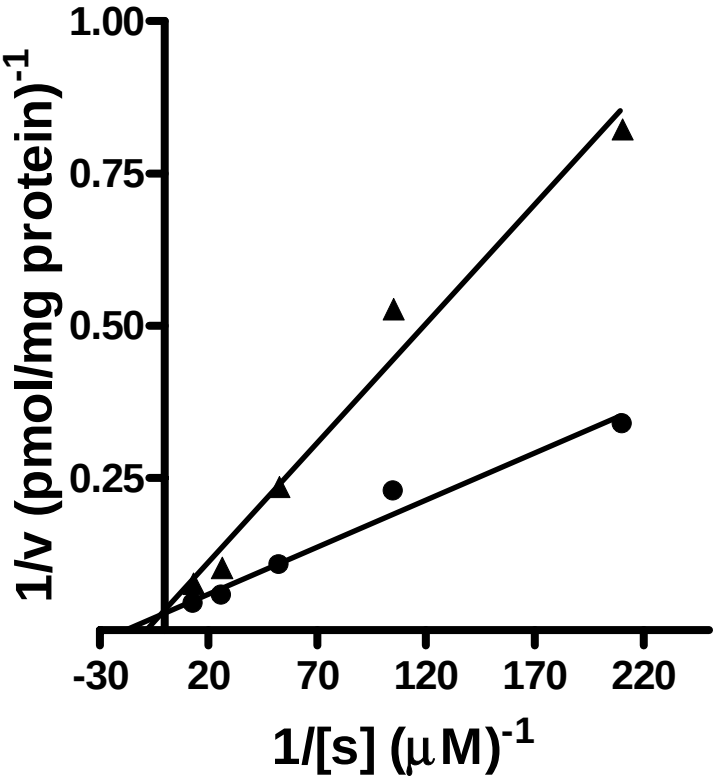

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Figure 5

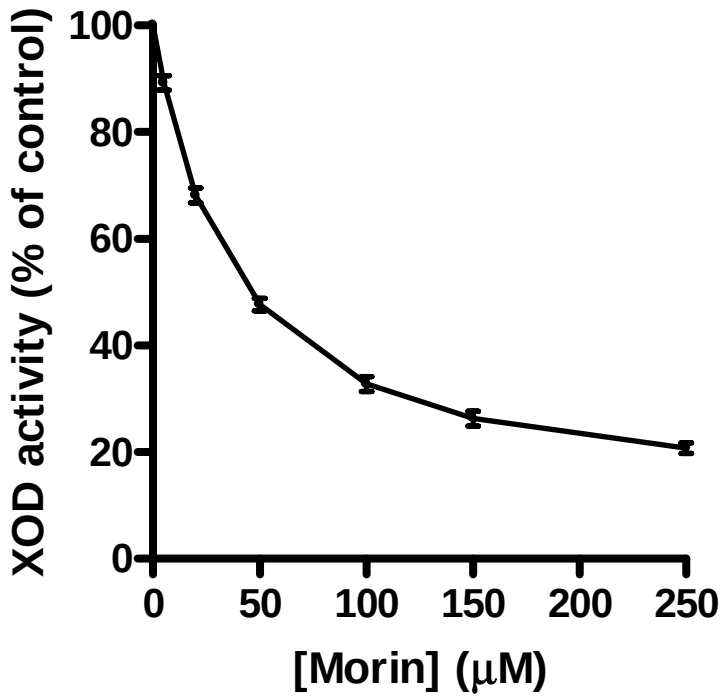

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Figure 6

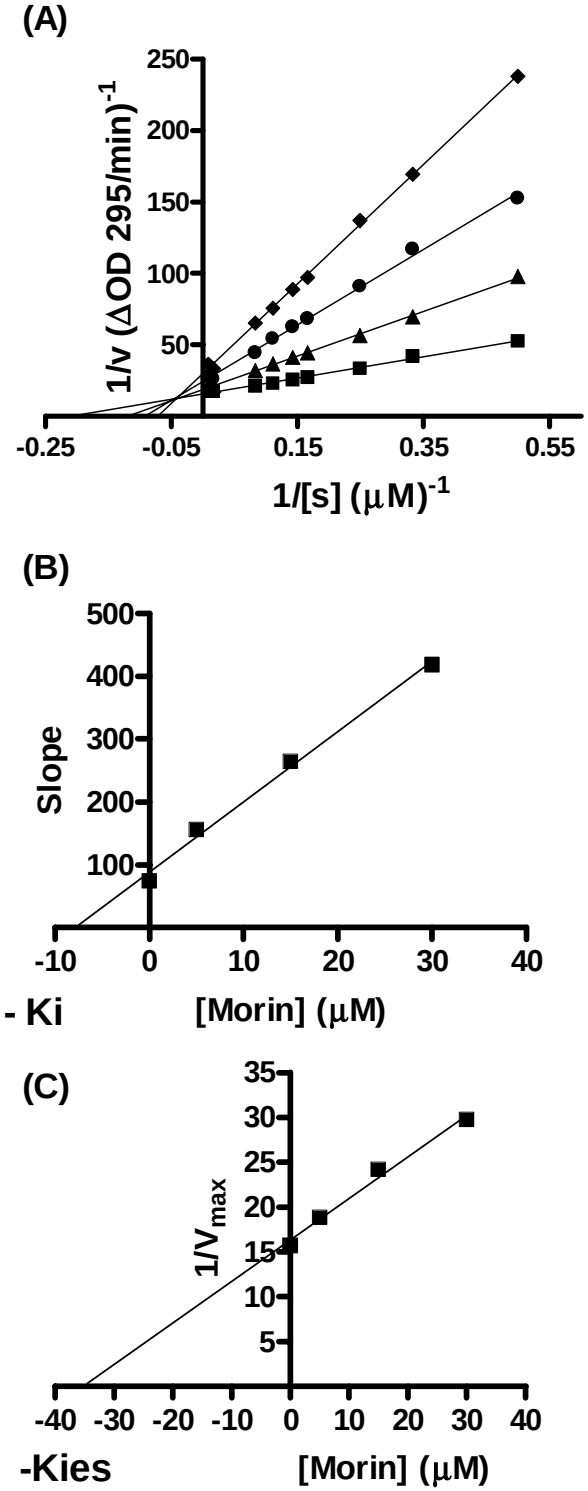

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Figure 7

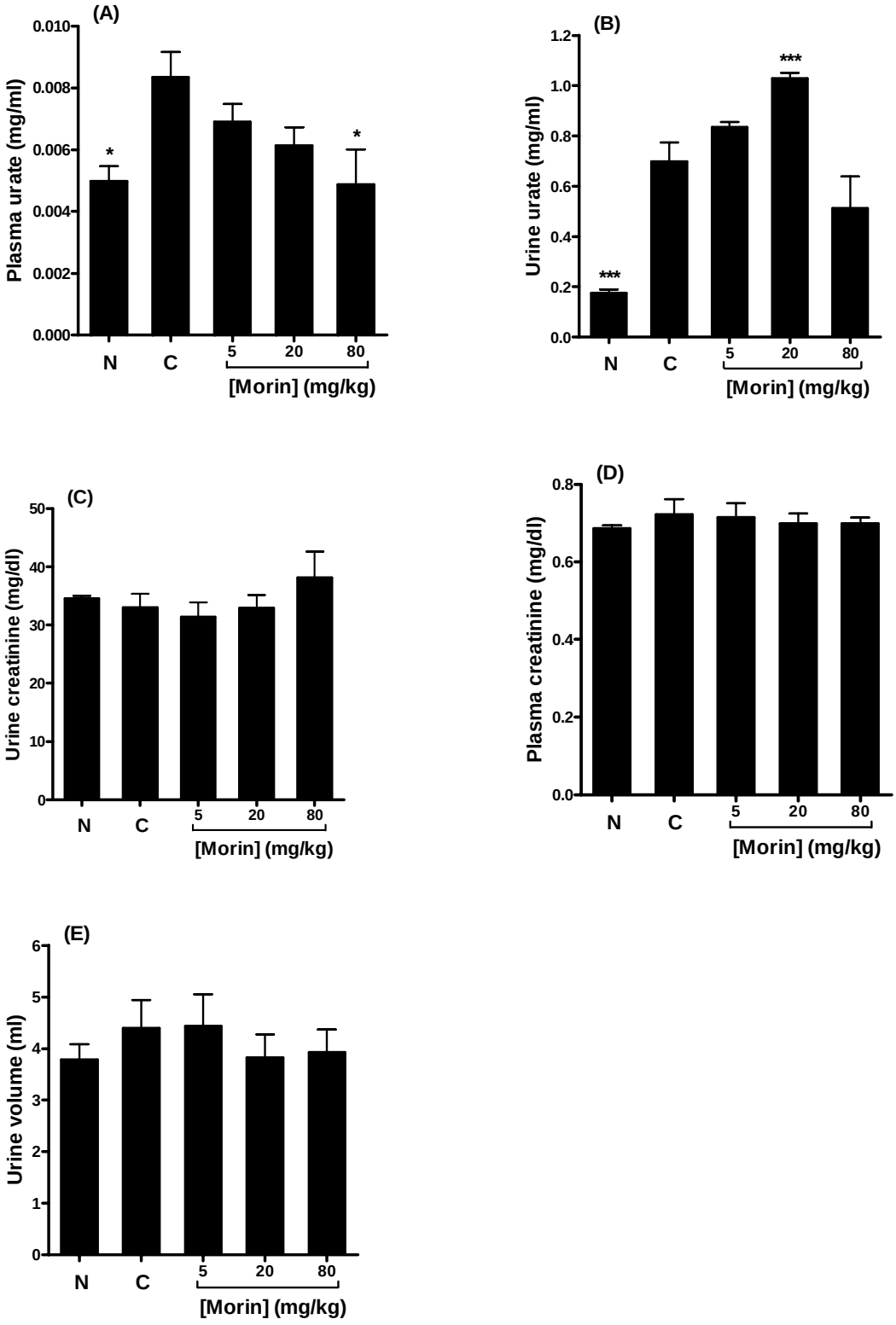

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Figure 8