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Effects of *Biota orientalis* extract and its flavonoid constituents, quercetin and rutin on serum uric acid levels in oxonate-induced mice and xanthine dehydrogenase and xanthine oxidase activities in mouse liver

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Abstract

The hypouricemic actions of *Biota orientalis* (BO) extract and its flavonoid constituents quercetin and rutin, were in vivo examined using oxonate-induced hyperuricemic mice. Quercetin and rutin, when administered three times orally to the oxonate-induced hyperuricemic mice, were able to elicit dose-dependent hypouricemic effects. The effects of quercetin and rutin were more potent than that of *Biota orientalis* extract at the same dose of 100 mg/kg. At doses of 50 mg/kg of quercetin or above, or at doses of 100 mg/kg of rutin or above, the serum urate levels of the oxonate-pretreated mice were not different from normal mice. In addition, *Biota orientalis* extract, quercetin and rutin, when tested in vivo on mouse liver homogenates, elicited significant inhibitory actions on the xanthine dehydrogenase/xanthine oxidase (XDH/XO) activities. The effects of quercetin and rutin resulted less potent than that of allopurinol. However, intraperitoneal administration at the same scheme did not produce any observable hypouricemic effect. These hypouricemic effects are partly due to the inhibition of XDH/XO activities in mouse liver. The pharmacological profile of the flavonoids is partly different from that of allopurinol. Such hypouricemic action and inhibition of the enzyme activity of quercetin and rutin may be responsible for a part of the beneficial effects of *Biota orientalis* extract on hyperuricemia and gout. The effects of quercetin and rutin on serum urate levels in hyperuricemic mice induced by oxonate and the inhibition of enzyme activities in mouse liver are discussed in relation to their absorption and metabolism, and their potential application to treat gout and hyperuricemia.

Keywords: *Biota orientalis*; Quercetin; Rutin; Hyperuricemic mice; Serum uric acid levels; Xanthine dehydrogenase/xanthine oxidase activities

1. Introduction

Gout is a common disease with a worldwide distribution and continues to be a health problem (Klemp et al., 1997; Arromdee et al., 2002; Liote, 2003; Zeng et al., 2003). Clinically reported, the key factor uric acid is related not only to an increased risk of gout, but also to an increased risk of cardiovascular disorder, nephrolithiasis and diabetes (Nakanishi et al., 1999; Chen et al., 2001; Kuzuya et al., 2002; Shekarriz and Stoller, 2002; Spieker et al., 2002). Nowadays, it seems to be widely accepted that the control of the key factor may also be considered in the prevention and treatment of these diseases (Liote, 2003). Xanthine dehydrogenase and xanthine oxidase (XDH/XO) is a complex metallo-flavoprotein. It catalyzes oxypurines (hypoxanthine and xanthine) to uric acid in the purine catabolic pathway. Inhibition of XDH/XO activities decreases the uric acid levels, and results in an anti-hyperuricemic effect. Allopurinol remains to be a dominant urate-lowering agent, however, adverse effects limit its therapy (Wallach, 1998).

Although the use of medicinal plants in the prevention and treatment of hyperuricemia and gout is based on the experience of traditional medicine systems (Theoduloz et al., 1988; Chiang et al., 1994; Guerrero and Guzman, 1998; Owen and Johns, 1999; Kong et al., 2000), their uses in modern medicine suffer from the lack of scientific evidences. Attention has been focused on identifying their phytochemicals, which possess ability to inhibit XDH/XO activities and thereby reduce the urate levels.

The leaves of *Biota orientalis* (L.) Endl. (Cupressaceae) have been used in Chinese medicine for treatments of gout, rheumatism, diarrhoea and chronic tracheitis (Jiangsu

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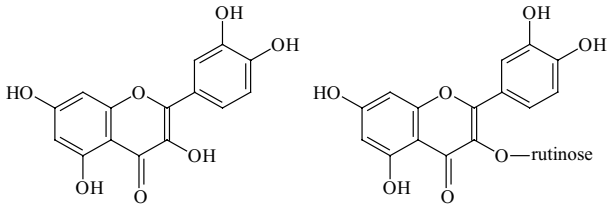

Fig. 1. Structures of quercetin and rutin.

College of New Medicine, 1977). Our unpublished preliminary test for XO inhibition showed that EtOH extract of the species possessed in vitro inhibitory effects against XO in mouse liver. This is an important indication that it might reduce serum uric acid levels by acting as the enzyme inhibitor. *Biota orientalis* (BO) consists of many chemical constituents such as flavonoids (Jiangsu College of New Medicine, 1977; Koo et al., 2002; Natarajan et al., 1970; Pelter et al., 1970; Xue et al., 1999), and one or more of these constituents may be effective agents as enzyme inhibitors. Flavonoids have been shown to be inhibitors of the activity of XO in in vitro study (Nagao et al., 1999). In this study, therefore, we have examined the efficacy of *Biota orientalis* extract, and its main flavonoid constituents quercetin and rutin (Fig. 1) in reducing serum urate levels in a mouse model of hyperuricemia induced by the uricase inhibitor potassium oxonate and in vivo inhibiting XDH/XO activities in mouse liver.

2. Materials and methods

2.1. Materials

The leaves of *Biota orientalis* (L.) Endl. (Cupressaceae), harvested in April 1998 in Sichuan, China, were purchased from the Jiangsu Herbal Drug Company, Nanjing, China. The material was sorted and identified by Ass. Prof. L.X. Zhang. A voucher specimen was deposited under the number NU-355333 in the herbarium of Nanjing University, Nanjing, China.

Biota orientalis (500 g) were twice refluxed with 60% EtOH for 1 h. The EtOH extract was filtered and concentrated to remove EtOH at 50 °C under vacuum. The EtOH extract was partitioned with petroleum to delete lipid-soluble substances, and then lypophilized into powder (yield 34.10 g, 6.82%). The main flavonoid constituents of *Biota orientalis* used in this study were identified as quercetin and rutin by comparing their R_f values of TLC and RT of HPLC with those of the authentic samples. The contents of quercetin and rutin in *Biota orientalis* extract were 1.76 and 3.08% by HPLC (Lopez et al., 2001). Quercetin, rutin, allopurinol, xanthine, nicotinamide adenine dinucleotide (NAD^+) and uric acid were purchased from Sigma Chemicals (St. Louis, MO, USA). Potassium oxonate was purchased from Aldrich Chemical Company, Inc. All other chemicals were the highest analytic grade available.

2.2. Animals

Male ICR mice (26–30 g) were purchased from the Laboratory Animal Center (Nanjing, Jiangsu Province, China) and were housed in plastic cages. They were allowed one week to adapt to their environment before used for experiments. All the animals were maintained on a 12-h light/12-h dark cycle, at a constant temperature of 25 °C. They are given standard chow and water ad libitum for the duration of the study. All the procedures were in strict accordance with the PR China legislation on the use and care of laboratory animals and with the guidelines established by Institute for Experimental Animals of Nanjing University and were approved by the university committee for animal experiments.

2.3. Animal model of hyperuricemia in mice

Experimental animal model of hyperuricemia induced by uricase inhibitor potassium oxonate has been used to study drug action (Stavric et al., 1975; Hall et al., 1990). Briefly, mice were injected intraperitoneally with potassium oxonate (280 mg/kg) 1 h before the final drug administration to increase the serum urate level. Whole blood samples were collected from mice by tail vein bleeding or cardiac puncture. The blood was allowed to clot for approximately 1 h at room temperature and then centrifuged at 2500 × *g* for 10 min to obtain the serum. The serum was stored at −20 °C until assayed. Serum uric acid was determined by the phosphotungstic acid method (Carroll et al., 1971).

2.4. Drug administration

Food, but not water, was withdrawn from the animals 1.5 h prior to drug administration. *Biota orientalis* extract, quercetin, rutin and allopurinol at various concentrations were dissolved in propyleneglycol/water (50/50). The volume of the suspension administered was based on body weight measured immediately prior to each dose, respectively. All drugs were given orally or intraperitoneally once daily at 14:00–15:00 h.

As showed in Fig. 2, the six groups (vehicle control and hyperuricemic control) were orally administered with 0.9% saline solution for 1, 3 and 7 days, respectively. The other 12 groups were orally treated with *Biota orientalis*, quercetin and rutin at 100 mg/kg and allopurinol at 10 mg/kg for 1, 3 and 7 days, respectively. As showed in Fig. 3, the two groups (vehicle control and hyperuricemic control) were intraperitoneally administered with 0.9% saline solution for 3 days, respectively. The other four groups were intraperitoneally received *Biota orientalis* extract, quercetin and rutin at 100 mg/kg and allopurinol at 10 mg/kg for 3 days, respectively. As showed in Table 1, the two groups (vehicle control and hyperuricemic control) were orally administered

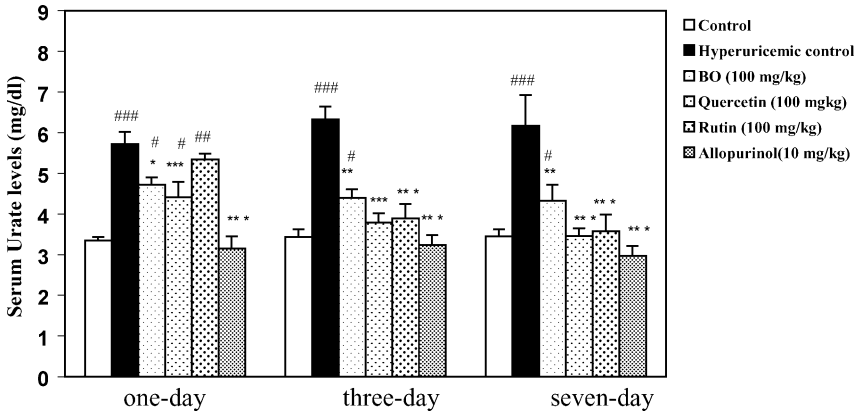

Fig. 2. Comparison of the effects of *Biota orientalis* extract, quercetin, rutin and allopurinol on serum urate levels in hyperuricemic mice pretreated with the potassium oxonate: a time-course study. The hyperuricemic mice were produced by potassium oxonate pretreatment as described in Section 2. They were then orally administered with *Biota orientalis* extract, quercetin, rutin or allopurinol at the different doses indicated, for 1, 3 or 7 days, respectively. The two control groups were dosed with vehicle instead. Data represent mean values (±S.E.M.) of serum urate levels (mg/dl) in the groups of animals ($n = 10$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. hyperuricemic control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. control group.

with 0.9% saline solution for 3 days. The other 12 groups were orally received quercetin and rutin at 25, 50, 100 and 150 mg/kg, allopurinol at 5, 10, 15 and 20 mg/kg for 3 days, respectively. As showed in Table 2, the one group (vehicle control) was orally administered with 0.9% saline solution for 3 days. The other eight groups were orally received *Biota orientalis* extract at 100 and 200 mg/kg, quercetin and rutin at 50 and 100 mg/kg, allopurinol at 5 and 10 mg/kg for 3 days, respectively.

2.5. Assays of XDH/XO activities

The enzyme activity assay started 1 h after final administration. Mouse liver was excised and homogenized in 5 volumes of 80 mM sodium pyrophosphate buffer (pH 7.4). The homogenate was then centrifuged at 3000 × *g* for 10 min, the lipid layer was carefully removed, and resulting supernatant fraction was further centrifuged at 10,000 × *g* for 60 min at 4 °C. The supernatant was used for enzyme assays.

The activities of XDH/XO were assayed by monitoring uric acid formation using a spectrophotometric method described in the previous papers (Hall et al., 1990; Kong et al., 2002). The reaction mixtures contained 50 μM phosphate buffer (pH 7.5), 100 μl liver homogenate, and 1 mM potassium allantoxanate, to avoid oxidation of uric acid to allantoin, in a final volume of 5.5 ml. For the assay of XDH activity, but not of XO activity, 200 μM NAD^{+} was also present. After preincubation for 15 min at 37 °C, the reaction was initiated by the addition of 1.2 ml of 250 μM xanthine. After 10 min, the reaction was stopped by the addition of 0.5 ml of 0.58 M HCl. The solution was then centrifuged at 3000 × *g* for 5 min, the supernatant were measured at 295 nm UV absorbance. XDH/XO activities were expressed as nanomole per minute per milligram protein. Each assay was performed in triplicate.

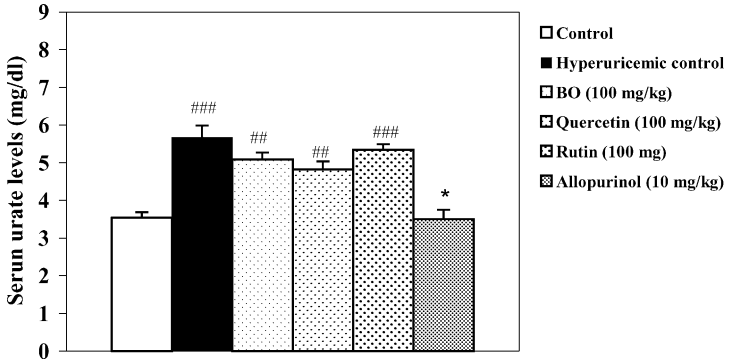

Fig. 3. Effect of intraperitoneal administration of *Biota orientalis* extract, quercetin, rutin and allopurinol on serum urate levels in hyperuricemic mice pretreated with the potassium oxonate. The hyperuricemic mice were produced by potassium oxonate pretreatment as described in Section 2. They were intraperitoneal administered with *Biota orientalis* extract, quercetin, rutin or allopurinol at the different doses indicated, for 3 days, respectively. The two control groups were dosed with vehicle instead. Data represent mean values (±S.E.M.) of serum urate levels (mg/dl) in the groups of animals ($n = 10$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. hyperuricemic control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. control group.

Table 1
Dose-dependent hypouricemic effects of quercetin and rutin on serum urate levels in mice pretreated with uricase inhibitor potassium oxonate after oral three-time administration

Treatment	Animals	Dose (mg/kg)	Serum urate levels (mg/dl)
Vehicle	12	–	3.14 ± 0.12
Hyperuricemia	12	–	5.03 ± 0.19$^{\#\#\#}$
Quercetin	12	25	4.37 ± 0.18$^{*,\#\#}$
	12	50	3.77 ± 0.14**
	12	100	3.52 ± 0.08***
	12	150	3.21 ± 0.11***
Rutin	12	25	4.97 ± 0.23$^{\#\#\#}$
	12	50	4.07 ± 0.14$^{*,\#}$
	12	100	3.83 ± 0.11***
	12	150	3.41 ± 0.13***
Allopurinol	10	5	4.71 ± 0.14$^{*,\#\#}$
	10	10	3.87 ± 0.16**
	10	15	3.43 ± 0.08***
	10	20	2.95 ± 0.14***

Data represent mean ± S.E.M. of 10 or 12 animals. For statistical significance, Student's *t*-test was used between control and drug groups. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, vs. hyperuricemia control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, versus control group.

2.6. Statistical analysis

Results shown represent the mean ± standard error of the mean (S.E.M.). The statistical evaluation of the results was carried out utilizing two-tailed, paired Student's *t*-tests. Statistical significance was set at $P < 0.05$.

3. Results

3.1. Effects of Biota orientalis extract, quercetin and rutin on serum urate levels in hyperuricemic mice

Uricase inhibitor potassium oxonate treatment caused hyperuricemia in mice, as indicated by drastic increases in serum uric acid levels. As shown in Fig. 2, after oral 1 day administration, *Biota orientalis* extract significantly reduced the urate levels and at the same time, quercetin, but not rutin, significantly reduced the urate levels, when compared with hyperuricemic control group. The action of quercetin on the urate levels was more potent than that of *Biota orientalis* extract. At the same dose pretreatment for three and seven times, quercetin and rutin showed to markedly reduce the urate levels in comparison to the hyperuricemic control group. The effects of quercetin and rutin were more potent than that of *Biota orientalis* extract. Furthermore, the serum urate levels of oxonate-pretreated mice dosed with quercetin and rutin for 3 days or 7 days were not significantly different from each other as well as from the control normal mice. These data indicated that three-time pretreatments of quercetin and rutin were needed for a complete recovery. The oral pretreatment of allopurinol at 10 mg/kg, elicited significant reduction of serum urate levels in the hyperuricemic mice to the normal value. Comparison showed that the difference in potency between quercetin, rutin and allopurinol appeared to be not significant in this case for three or seven-time pretreatment, respectively.

3.2. Dose-dependent effects of quercetin and rutin on serum urate levels in hyperuricemic mice

Quercetin and rutin were more potent in decreasing serum urate levels in hyperuricemic mice than *Biota orientalis* extract for three-time pretreatment. Therefore, in further experiments, the dose-dependence of quercetin and rutin on the urate levels was examined. As shown in Table 1, orally treating hyperuricemic mice with quercetin at a daily dose of 25–150 mg/kg for three times, produced dose-dependent decreases in the levels. At doses of 50 mg/kg of quercetin or above, the levels of the oxonate-pretreated mice were not different from the normal value. Pretreatments with rutin (25–150 mg/kg) also caused dose-dependent reduction in serum urate levels in hyperuricemic mice. At doses of 100 mg/kg of rutin or above, the levels in hyperuricemic

Table 2
Effects of *Biota orientalis* extract, quercetin and rutin on xanthine dehydrogenase (XDH) and xanthine oxidase (XO) activities in mouse liver after oral three-time administration

Treatment	Dose (mg/kg)	XDH (nanomole uric acid per minute per milligram protein)	XO (nanomole uric acid per minute per milligram protein)	% Inhibition	
				XDH	XO
Vehicle	–	7.63 ± 0.45	4.48 ± 0.20	–	–
Biota orientalis	100	4.32 ± 0.47**	4.26 ± 0.31	43.38	4.90
	200	4.08 ± 0.78**	3.87 ± 0.35*	46.52	13.62
Quercetin	50	4.04 ± 0.71**	4.00 ± 0.45	47.05	10.71
	100	3.51 ± 0.36**	3.69 ± 0.31*	54.00	17.63
Rutin	50	4.40 ± 0.83**	4.04 ± 0.41	42.33	9.82
	100	3.59 ± 0.35**	3.81 ± 0.33*	52.94	14.96
Allopurinol	5	3.97 ± 0.34***	2.95 ± 0.17**	47.96	34.15
	10	2.18 ± 0.34***	2.74 ± 0.55***	71.42	38.83

Data represent mean ± S.E.M. of 10 animals. For statistical significance, Student's *t*-test was used between vehicle control and drug groups. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, vs. vehicle.

mice were not different from the normal value. Allopurinol at a daily dose of 5–20 mg/kg significantly reduced the urate levels in hyperuricemic mice at dose-dependent manner.

3.3. Effects of Biota orientalis extract, quercetin and rutin on XDH/XO activities in mouse liver

As shown in Table 2, treating mice with *Biota orientalis* extract at a daily dose of 100 and 200 mg/kg for 3 days caused inhibition by 43.38 and 46.52% towards XDH, 4.90 and 13.62% towards XO, when compared with the vehicle group, respectively. Quercetin, at the doses of 50 and 100 mg/kg, showed inhibition by 47.05 and 54.00% towards XDH, 10.71 and 17.63% towards XO. Rutin pretreatments at 50 and 100 mg/kg inhibited enzyme activities in mouse liver, with inhibition by 42.33 and 52.94% towards XDH, 9.82 and 14.96% towards XO. The inhibitions of the XDH/XO activities of rutin resulted similar to that of quercetin. On the other hand, the actions of quercetin and rutin on the inhibitions of XDH/OX activities were more potent than that of *Biota orientalis* extract at the same dose of 100 mg/kg. Allopurinol inhibited both XDH (47.96 and 71.42% inhibition) and XO (34.15 and 38.83% inhibition) activities at the doses of 5 and 10 mg/kg, respectively, exhibiting to be more potent than flavonoids in the XO inhibition.

3.4. Effects of intraperitoneal administration of Biota orientalis extract, quercetin and rutin on serum urate levels in hyperuricemic mice

As shown in Fig. 3, after the three-time pretreatment via the peritoneal route, *Biota orientalis* extract and quercetin, but not rutin at the same dose of 100 mg/kg produced a slight, but not significant decrease in serum urate level in oxonate-treated mice. In contrast, allopurinol at 10 mg/kg was active in this experiment.

3.5. Effects of Biota orientalis extract, quercetin and rutin on serum urate levels in normal mice

The same pretreatment scheme of *Biota orientalis* extract, quercetin and rutin via the peritoneal and oral route did not produce any remark change in serum urate levels in normal mice (data not shown). Allopurinol significantly reduced the levels in normal mice treated with the same administration (data not shown).

4. Discussion

Gout is one of the most common metabolic disorders in humans, reportedly afflicts more than two million men and women in the United States. It is characterized by marked hyperuricemia, caused the deposition of urate monohydrate crystals in joint and kidney, resulting in gouty arthritis and uric acid nephrolithiasis (Kramer and Curhan, 2002). The increased risk of hyperuricemia has been also linked with the development of hypertension and hyperlipidemia (two cardiovascular risk factors) (Emmerson, 1998), cancer (Garcia-Porrua et al., 1999), diabetes, hypertension and obesity (Chen et al., 2001). Uric acid level is the key factor for prevention of gout and other disorders (Lin et al., 2000). In previous years many authors have shown a growing interest for the protective role of XO inhibitor allopurinol on urate levels of hyperuricemia and gout. However, it has been observed that allopurinol induce side effects such as hypersensitivity syndrome (Hammer et al., 2001), Stevens–Johnson syndrome (Fritsch and Sidoroff, 2000) and renal toxicity (Horiuchi et al., 2000). An alternative to allopurinol is represented by some phytochemicals like flavonoids. Flavonoids are naturally occurring plant compounds with XO inhibitory properties (Nagao et al., 1999). Furthermore, their consumption has been associated with the protective effects of certain diets and herbs against some of the complications of hyperuricemia and gout, such as cardiovascular disease and diabetes (Kimira et al., 1998; Sampson et al., 2002). In fact, quercetin and rutin have been reported to possess a wide range of biological activities (Autore et al., 2001; da Silva et al., 2002; Shen et al., 2002; Gerhauser et al., 2003; Moon et al., 2003). It is therefore generally recognized that they appear to be cost effective in hyperuricemic and gouty patients with these disorders.

The extract of *Biota orientalis* is being frequently used for the treatment of gout, and rheumatis (Jiangsu College of New Medicine, 1977). Previously, this species was disclosed to be the rich source of flavonoids such as quercetin and rutin (Jiangsu College of New Medicine, 1977; Natarajan et al., 1970; Pelter et al., 1970). We have previously shown that *Biota orientalis* extract exhibited in vitro pronounced inhibitions against XO from mouse liver and decreased the urate level in hyperuricemic mice. Pretreatment with the flavonoid constituents of *Biota orientalis* extract also decreased the urate levels in the animal model. Among the flavonoid constituents examined, quercetin seemed to be more effective in reducing the urate levels than did *Biota orientalis* extract after oral administration. Furthermore, both flavonoid constituents were very significantly effective in decreasing the urate levels after three or seven-time oral administration. These results suggested that quercetin and rutin were capable of reducing the accumulation of purine metabolites in blood following oxonate-induction.

It seems likely that these flavonoids reduce serum urate levels by inhibiting XDH/XO activities. In fact, it has been shown that flavonoids could inhibit the formation of uric acid from xanthine by XO in vitro (Nagao et al., 1999; Selloum et al., 2001). Thus, there is a possibility that the flavonoids at the same dose scheme may in vivo inhibit the XDH/XO activities in mouse liver. In our present study, we observed that *Biota orientalis* extract, quercetin and rutin in vivo exhibit inhibitory actions on enzyme activities in mouse liver,

preferentially inhibiting XDH activity over XO activity. Although the potency of the flavonoids at 100 mg/kg on the inhibition of the XDH/XO activities was less than that of allopurinol at the dose of 10 mg/kg, the action of the flavonoids on the reduction of the urate levels in hyperuricemic mice was similar to that of allopurinol, after oral three-time administration. We treated normal mice with *Biota orientalis* extract and the flavonoids with the aim to evaluate possible allopurinol-like effect. While allopurinol caused a decrease in the urate levels, treatment with *Biota orientalis* extract, quercetin and rutin, did not cause any significant change in the levels, indicating that *Biota orientalis* extract, and these flavonoids did not have allopurinol-like hypouricemic effects influencing uric acid levels in the normal purine metabolic system. The in vivo hypouricemic effects produced by *Biota orientalis* extract, quercetin and rutin seem to be partly mediated by the inhibition of XDH/XO activity. They could be used alternatively to allopurinol in prevention of hyperuricemic disorders. We have also examined the presence of quercetin and rutin in *Biota orientalis* extract by TLC and HPLC. However, the levels of quercetin and rutin were low in the extract, there is a possibility that quercetin and rutin may partly contribute to the beneficial effects of *Biota orientalis* extract on the reductions of the urate levels and the inhibition of enzyme activities. Further investigations are warranted to identify the other active principle(s) of the *Biota orientalis* extract, responsible for the observed hypouricemic effects.

Generally, the drug bioactivity in vivo is dependent on absorption and metabolism. Quercetin is an aglycon flavonol. It mainly occurs in plants in the form of glycosides, such as rutin (quercetin-3-*O*-beta-rutinoside). Several experimental and clinical studies have suggested that overall kinetic behaviour of quercetin differed remarkably after the ingestion of rutin. Interestingly, in the present study, it was found that after one-, but not three-time oral pretreatment at the dose of 100 mg/kg, quercetin showed hypouricemic effect, whereas rutin did not have the action. The result suggested that quercetin was absorbed more quickly than rutin. It is consistent with the finding that quercetin was rapidly absorbed by the stomach. In contrast, its glycosidic form rutin was not hydrolyzed and not absorbed by this tissue in the rats (Crespy et al., 2002). Furthermore, pharmacokinetic studies have shown that the small intestine is the major site of absorption for many flavonoid glucosides. Quercetin and rutin could be absorbed at different segments of intestine, but the absorption of rutin was significantly lower than that of quercetin (Su et al., 2002). Time to reach C_{max} (t_{max}) was significantly shorter after the quercetin aglycone treatment than after the rutin treatment (Erlund et al., 2000). Recently, there is an increasing evidence that the absorption of flavonoid glycosides in humans involves a critical deglycosylation step that is mediated by epithelial beta-glucosidases (Nemeth et al., 2003). Clinically previous investigations also indicate that the disposition of quercetin in humans primarily depends on the sugar moiety. The site of absorption seems to be different from rutin (Graefe et al., 2001). These results are in agreement with the earlier reported studies of Morand et al. (2000), who showed that the nature of the glycosylation markedly influences the efficiency of quercetin absorption in rats. The binding of a rhamnose or of a glucose-rhamnose moiety to the aglycone markedly depressed its absorption. Quercetin inhibited the formation of uric acid from xanthine by XO, while rutin was ineffective in vitro (Selloum et al., 2001). Thus, it might be possible that the high circulating levels of the active forms of rutin such as glucuronidated, sulphated and methylated derivatives reaching the systemic circulation lead to the decrease in serum uric acid levels and the inhibition in enzyme activities (Boyle et al., 2000; Kessler et al., 2003). Further investigation is warranted.

It was reported that intraperitoneal administration of some flavonoids such as rutin significantly reduced small and large intestinal transit in mice (Di Carlo et al., 1993). The compounds via oral route have both intestinal absorption and first pass effect through the liver, and the amounts of active constituents in the intestine and liver via oral route may be higher than that via intraperitoneal route because of their different absorption. Furthermore, it is well-known that xanthine oxidase in the small intestine and liver is at high levels in many mammals (Krenitsky et al., 1986). In the present study, at the same doses, when *Biota orientalis* extract, quercetin and rutin were intraperitoneally administered to the hyperuricemic mice, they did not elicit any significant hypouricemic effect. This lack of effect via the peritoneal route might be due to small and large intestinal transit reduction and low concentrations of active constituents in the intestine and liver. Future studies will still be continued to elucidate on the exact mechanism of the action of the bioactive forms of *Biota orientalis* extract, quercetin and rutin in vivo.

In conclusion, the data reported in the present study indicate that orally administered *Biota orientalis* extract, quercetin and rutin reduce serum urate levels of hyperuricemic mice caused by oxonate. These hypouricemic effects are partly due to inhibition of XDH/XO activities in mouse liver. The pharmacological profile of quercetin and rutin is partly different from that of allopurinol. Quercetin and rutin may be partly responsible for the hypouricemic actions of *Biota orientalis* extract. It is therefore suggested that *Biota orientalis* extract and the flavonoids may represent a new type of hypouricemic agents and they may have a potent hypouricemic effect in clinical use.

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References

Arromdee, E., Michet, C.J., Crowson, C.S., O'Fallon, W.M., Gabriel, S.E., 2002. Epidemiology of gout: is the incidence rising? Journal of Rheumatology 29, 2403–2406.

Autore, G., Rastrelli, L., Lauro, M.R., Marzocco, S., Sorrentino, R., Sorrentino, U., Pinto, A., Aquino, R., 2001. Inhibition of nitric oxide synthase expression by a methanolic extract of Crescentia alata and its derived flavonols. Life Science 70, 523–534.

Boyle, S.P., Dobson, V.L., Duthie, S.J., Hinselwood, D.C., Kyle, J.A., Collins, A.R., 2000. Bioavailability and efficiency of rutin as an antioxidant: a human supplementation study. European Journal of Clinical Nutrition 54, 774–782.

Carroll, J.J., Roberta Douglass, H.C., Babson, A.L., 1971. A simplified alkaline phosphotungstate assay for uric acid in serum. Clinical Chemistry 17, 158–160.

Chen, S.Y., Chen, C.L., Shen, M.L., Kamatani, N., 2001. Clinical features of familial gout and effects of probable genetic association between gout and its related disorders. Metabolism 50, 1203–1207.

Chiang, H.C., Lo, Y.J., Lu, F.J., 1994. Xanthine oxidase inhibitors from the leaves of Alsophila spinulosa (Hook) Tryon. Journal of Enzyme Inhibition 8, 61–71.

Crespy, V., Morand, C., Besson, C., Manach, C., Demigne, C., Remesy, C., 2002. Quercetin, but not its glycosides, is absorbed from the rat stomach. Journal of Agricultural and Food Chemistry 50, 618–621.

da Silva, J., Herrmann, S.M., Heuser, V., Peres, W., Possa Marroni, N., Gonzalez-Gallego, J., Erdtmann, B., 2002. Evaluation of the genotoxic effect of rutin and quercetin by comet assay and micronucleus test. Food and Chemical Toxicology 140, 941–947.

Di Carlo, G., Autore, G., Izzo, A.A., Maiolino, P., Mascolo, N., Viola, P., Diurno, M.V., Capasso, F., 1993. Inhibition of intestinal motility and secretion by flavonoids in mice and rats: structure-activity relationships. Journal of Pharmacy and Pharmacology 45, 1054–1059.

Emmerson, B., 1998. Hyperlipidaemia in hyperuricaemia and gout. Annuals of the Rheumatic Diseases 57, 509–510.

Erlund, I., Kosonen, T., Alfthan, G., Maenpaa, J., Perttunen, K., Kenraali, J., Parantainen, J., Aro, A., 2000. Pharmacokinetics of quercetin from quercetin aglycone and rutin in healthy volunteers. European Journal of Clinical Pharmacology 56, 545–553.

Fritsch, P.O., Sidoroff, A., 2000. Drug-induced Stevens-Johnson syndrome/toxic epidermal necrolysis. American Journal of Clinical Dermatology 1, 349–360.

Garcia-Porrua, C., Gonzalez-Gay, M.A., Vazquez-Caruncho, M., 1999. Tophaceous gout mimicking tumoral growth. Journal of Rheumatology 26, 508–509.

Gerhauser, C., Klimo, K., Heiss, E., Neumann, I., Gamal-Eldeen, A., Knauft, J., Liu, G.Y., Sitthimonchai, S., Frank, N., 2003. Mechanism-based in vitro screening of potential cancer chemopreventive agents. Mutation Research 523–524, 163–172.

Graefe, E.U., Wittig, J., Mueller, S., Riethling, A.K., Uehleke, B., Drewelow, B., Pforte, H., Jacobasch, G., Derendorf, H., Veit, M., 2001. Pharmacokinetics and bioavailability of quercetin glycosides in humans. Journal of Clinical Pharmacology 41, 492–499.

Guerrero, R.O., Guzman, A.L., 1998. Inhibition of xanthine oxidase by Puerto Rican plant extracts. PR Health Science Journal 17, 359–364.

Hall, I.H., Scoville, J.P., Reynolds, D.J., Simlot, R., Duncan, P., 1990. Substituted cyclic imides as potencial anti-gout agents. Life Sciences 46, 1923–1927.

Hammer, B., Link, A., Wagner, A., Bohm, M., 2001. Fatal allopurinol-induced hypersensitivity in asymptomatic hyperuricemia syndrome. Deutsch Medizinisch Wochenschrift 126, 1331–1334.

Horiuchi, H., Ota, M., Nishimura, S., Kaneko, H., Kasahara, Y., Ohta, T., Komoriya, K., 2000. Allopurinol induces renal toxicity by impairing pyrimidine metabolism in mice. Life Science 66, 2051–2070.

Jiangsu College of New Medicine, 1977. The Dictionary of the Traditional Chinese Medicine. Shanghai Press of Science and Technology, Shanghai, pp. 1375–1377.

Kessler, M., Ubeaud, G., Jung, L., 2003. Anti- and pro-oxidant activity of rutin and quercetin derivatives. Journal of Pharmacy and Pharmacology 55, 131–142.

Kimira, M., Arai, Y., Shimoi, K., Watanabe, S., 1998. Japanese intake of flavonoids and isoflavonoids from foods. Journal of Epidemiology 8, 168–175.

Klemp, P., Stansfield, S.A., Castle, B., Robertson, M.C., 1997. Gout is on the increase in New Zealand. Annuals of the Rheumatic Diseases 56, 22–26.

Kong, L.D., Cai, Y., Huang, W.W., Christopher, H.K., Cheng, C.H.K., Tan, R.X., 2000. Inhibition of xanthine oxidase by some Chinese medicinal plants used to treat gout. Journal of Ethnopharmacology 73, 199–207.

Kong, L.D., Zhou, J., Wen, Y.L., Li, J.M., Cheng, C.H.K., 2002. Aesculin possesses potent hypouricemic action in rodents but is devoid of xanthine oxidase/dehydrogenase inhibitory activity. Planta Medica 68, 175–178.

Koo, K.A., Sung, S.H., Kim, Y.C., 2002. A new neuroprotective pinusolide derivative from the leaves of Biota orientalis. Chemical and Pharmaceutical Bulletin (Tokyo) 50, 834–836.

Kramer, H.M., Curhan, G., 2002. The association between gout and nephrolithiasis: the National Health and Nutrition Examination Survey III 1988–1994. American Journal of Kidney Disease 40, 37–42.

Krenitsky, T.A., Spector, T., Hall, W.W., 1986. Xanthine oxidase from human liver: purification and characterization. Archives of Biochemistry and Biophysics 247, 108–119.

Kuzuya, M., Ando, F., Iguchi, A., Shimokata, H., 2002. Effect of aging on serum uric acid levels: longitudinal changes in a large Japanese population group. Journal of Gerontology Series A: Biological Sciences and Medical Sciences 57, 660–664.

Lin, K.C., Lin, H.Y., Chou, P., 2000. The interaction between uric acid level and other risk factors on the development of gout among asymptomatic hyperuricemic men in a prospective study. Journal of Rheumatology 27, 1501–1505.

Liote, F., 2003. Hyperuricemia and gout. Current Rheumatology Report 5, 227–234.

Lopez, M., Martinez, F., Del Valle, C., Orte, C., Miro, M., 2001. Analysis of phenolic constituents of biological interest in red wines by high-performance liquid chromatography. Journal of Chromatography A 13, 359–363.

Morand, C., Manach, C., Crespy, V., Remesy, C., 2000. Respective bioavailability of quercetin aglycone and its glycosides in a rat model. Biofactors 12, 169–174.

Moon, S.K., Cho, G.O., Jung, S.Y., Gal, S.W., Kwon, T.K., Lee, Y.C., Madamanchi, N.R., Kim, C.H., 2003. Quercetin exerts multiple inhibitory effects on vascular smooth muscle cells: role of ERK1/2, cell-cycle regulation, and matrix metalloproteinase-9. Biochemical and Biophysical Research Communications 301, 1069–1078.

Nagao, A., Seki, M., Kobayashi, H., 1999. Inhibition of xanthine oxidase by flavonoids. Bioscience, Biotechnology, and Biochemistry 63, 1787–1790.

Nakanishi, N., Suzuki, K., Kawashimo, H., Nakamura, K., Tatara, K., 1999. Serum uric acid: correlation with biological, clinical and behavioral factors in Japanese men. Journal of Epidemiology 9, 99–106.

Natarajan, S., Murti, V.V., Seshadri, S., Tiruvenkata, R., Biflavonoids, I.V., 1970. Bioflavones of some Cupressaceae plants. Phytochemistry 9, 575–579.

Nemeth, K., Plumb, G.W., Berrin, J.G., Juge, N., Jacob, R., Naim, H.Y., Williamson, G., Swallow, D.M., 2003. Deglycosylation by small intestinal epithelial cell beta-glucosidases is a critical step in the absorption and metabolism of dietary flavonoid glycosides in humans. European Journal of Nutrition 42, 29–42.

Owen, P.L., Johns, T., 1999. Xanthine oxidase inhibitory activity of northeastern North American plant remedies used for gout. Journal of Ethnopharmacology 64, 149–160.

Pelter, A., Warren, R., Hameed, N., Khan, N.U., Ilyas, M., Rahman, W., 1970. Bioflavonyl pigments from *Thuja orientalis*. Phytochemistry 9, 1897–1898.

Sampson, L., Rimm, E., Hollman, P.C., de Vries, J.H., Katan, M.B., 2002. Flavonol and flavone intakes in US health professionals. Journal of American Diet Association 102, 1414–1420.

Selloum, L., Reichl, S., Muller, M., Sebihi, L., Arnhold, J., 2001. Effects of flavonols on the generation of superoxide anion radicals by xanthine oxidase and stimulated neutrophils. Archives of Biochemistry Biophysics 395, 49–56.

Shekarriz, B., Stoller, M.L., 2002. Uric acid nephrolithiasis: current concepts and controversies. The Journal of Urology 168, 1307–1314.

Shen, S.C., Lee, W.R., Lin, H.Y., Huang, H.C., Ko, C.H., Yang, L.L., Chen, Y.C., 2002. In vitro and in vivo inhibitory activities of rutin, wogonin, and quercetin on lipopolysaccharide-induced nitric oxide and prostaglandin E(2) production. European Journal of Pharmacology 446, 187–194.

Spieker, L.E., Ruschitzka, F.L., Luscher, T.F., Noll, G., 2002. The management of hyperuricemia and gout in patients with heart failure. European Journal of Heart Failure 4, 403–410.

Stavric, B., Clayman, S., Gradd, R.E.A., Hebert, D., 1975. Some in vivo effects in the rat induced by chlorprothixene and potassium oxonate. Pharmacology Research Communication 7, 117–124.

Su, J., Guo, C., Wei, J., Yang, J., 2002. Study on the absorption of quercetin and rutin at different segments of intestine. Wei Sheng Yan Jiu 31, 55–57.

Theoduloz, C., Franco, L., Ferro, E., Hirschmann, G.S., 1988. Xanthine oxidase inhibitory activity of Paraguayan Myrtaceae. Journal of Ethnopharmacology 24, 179–183.

Wallach, S.L., 1998. The side effects of allopurinol. Hospital Practice (Off Ed) 33, 22.

Xue, W.M., Zhang, X.L., Kang, M.D., 1999. An applied study on measurement of general flavone of Cacumen Biotae by spectrophotometric determination combined with membrane-based. Journal of Northwest University (Natural Science Edition) 29, 37–40.

Zeng, Q., Wang, Q., Chen, R., Xiao, Z., Huang, S., Xu, J., 2003. Primary gout in Shantou: a clinical and epidemiological study. Chinese Medical Journal 116, 66–69 (in English).