別紙様式（Ⅴ）-２【添付ファイル用】

特定保健用食品とは異なる臨床試験方法とした合理的理由に関する説明資料

１．製品概要

商品名	ルテオリン　尿酸ダウン
機能性関与成分名	ルテオリン
表示しようとする機能性	本品にはルテオリンが含まれます。ルテオリンには尿酸値が高め（5.5 ㎎/dL 超～7.0 ㎎/dL 未満）な男性の尿酸値を下げる機能があります。

２．特定保健用食品とは異なる臨床試験方法（科学的合理性が担保されたものに限る。）とした合理的理由

本製品を用いた臨床試験の内容の一部について、「特定保健用食品の表示許可等について」（平成 26 年 10 月 30 日消食表第 259 号）の別添 2「特定保健用食品申請に係る申請書作成上の留意事項」に準拠していない形での方法を採用した。以下に科学的合理性が担保されていると判断した理由を記載する。

１.評価指標

「特定保健用食品申請に係る申請書作成上の留意事項」には、本製品の表示しようとする機能性に合致する保健の用途に係る有効性について記載がない。そこで、日本痛風・核酸代謝学会の「高尿酸血症・痛風の治療ガイドライン（第 2 版)」を参考に、ガイドライン内で高尿酸血症・痛風の診断基準となっている血中尿酸値を主要アウトカムとした試験を実施した。

２.被験者の特徴

本製品を用いた臨床試験では男性のみを対象としており、被験者に女性は含まれていない。一般的に男性に比べて女性は高尿酸血症および痛風の患者数が少なく、治療が必要な高尿酸血症患者のうち、女性の割合は 2.5%だったという報告もある[1)]。これは女性ホルモンであるエストロゲンが、腎臓内の尿酸の再吸収に関与するトランスポーターURAT1 の働きを抑制するためと言われている[2)]。これらの理由から、尿酸値を指標とした臨床試験では男性のみを対象としている例もあり[3, 4)]、本製品を用いた臨床試験の対象者を男性のみにしたことは妥当であると考えられる。

３．その他

本製品を用いた臨床試験は以下に示す内容で実施したことから、科学的合理性が担保できていると判断が可能である。

・試験デザインはランダム化二重盲検プラセボ対照比較試験とした
・試験品には本製品と同等の形状（ハードカプセル)、配合処方のものを使用しており、プラセボ食品は色や形状などの外観で試験品との識別ができないようにして行った。

以上の理由から、本製品を用いた臨床試験における科学的合理性は担保できていると判断した。

【参考文献】

1) 桑原政成ら，痛風と核酸代謝，36（1），p.72（2012）
2) MiClory J. and Said N. *Medicine and Health Rhole Island; Providence*, 92（11），363-364（2009）
3) Ueda H. et al., *NutraFoods*, 14, 151-158（2015）
4) 鎌谷直之ら，痛風と核酸代謝，40（1），p.21-31（2016）

別紙様式（Ⅴ）-３【添付ファイル用】

表示しようとする機能性の科学的根拠に関する補足説明資料

１．製品概要

商品名	ルテオリン　尿酸ダウン
機能性関与成分名	ルテオリン
表示しようとする機能性	本品にはルテオリンが含まれます。ルテオリンには尿酸値が高め（5.5 ㎎/dL 超～7.0 ㎎/dL 未満）な男性の尿酸値を下げる機能があります。

２．補足説明

＜臨床試験に用いた試験品と本製品の同等性について＞

本製品は臨床試験に用いた試験品と全く同じ配合処方、サプリメント形態（ハードカプセル）で製造されている。また、本製品と試験品に配合した機能性関与成分「ルテオリン」を含む菊の花抽出物（菊の花エキス）は、どちらも全く同じ製法、製造場所、抽出溶媒および菊花の品種で製造されており、機能性関与成分の含有量も同等であり、ルテオリンは分子量 286 で示される単一成分である。よって、本製品と臨床試験に用いた試験品にはルテオリンの含有量が同等量（1 日推奨摂取量として 10 mg）配合されている。

以上のことから、本製品を摂取することにより、臨床試験によって示された機能性と同一の機能性が示されると考えられる。

＜層別解析について＞

本製品を用いた臨床試験において、摂取前後の尿酸値の変化量をプラセボ群と比較した結果、試験品摂取群とプラセボ群との間に有意差は認められなかった（表 1）。しかしながら、ベースラインにおいて血中尿酸値が 5.5 mg/dL 超～7.0 mg/dL 未満（血清尿酸値正常範囲[1)]）の被験者を抽出して層別解析を行った結果、変化量におけるプラセボ群との比較において、試験品摂取群の有意な尿酸値低下が認められた（表 2）。

以上のことから、本製品の表示しようとする機能性を「本品にはルテオリンが含まれます。ルテオリンには尿酸値が高め（5.5 ㎎/dL 超～7.0 ㎎/dL 未満）な男性の尿酸値を下げる機能があります。」と表示することには合理的根拠があると判断した。

表 1. プラセボ及び試験品を 4 週間摂取させた時の血中尿酸値の変化

		摂取前	摂取後	変化量
血中尿酸値 (mg / dL)	プラセボ群	5.96 ± 1.10	5.85 ± 0.96	-0.11 ± 0.54
	試験品群	6.00 ± 1.00	5.82 ± 0.97 †	-0.18 ± 0.37

それぞれの数値は平均値±標準偏差を示している。有意差検定は、摂取前後の絶対値の比較および変化量の比較において、paired *t*-test を用いた。またプラセボ群と試験品群の比較においても同様に paired *t*-test を用いた。摂取前後の有意差は†: $P < 0.05$ にて示した。

表 2. ベースラインの尿酸値が 5.5 mg/dL 超～7.0 mg/dL 未満の被験者における、プラセボ及び試験品を 4 週間摂取させた時の血中尿酸値の変化

		摂取前	摂取後	変化量
血中尿酸値 (mg / dL)	プラセボ群	5.91 ± 0.59	6.09 ± 0.52	0.18 ± 0.48
	試験品群	6.18 ± 0.65	5.98 ± 0.55	-0.20 ± 0.38 *

それぞれの数値は平均値±標準偏差を示している。有意差検定は、摂取前後の絶対値の比較および変化量の比較において、paired *t*-test を用いた。またプラセボ群と試験品群の比較においても同様に paired *t*-test を用いた。群間の有意差は *: $P < 0.05$ にて示した。

＜高めの尿酸値の定義付けについて＞

本製品を用いた臨床試験では、血中尿酸値 5.5 mg/dL 超～7.0 mg/dL 未満を健常範囲で高めの尿酸値として層別解析を行っている。日本痛風・核酸代謝学会による痛風治療ガイドラインによると、男性の場合、尿酸値が 7.0 mg/dL を超えると高尿酸血症と診断される[1)]。この高尿酸血症は治療の対象であるため、血中尿酸値の正常範囲の上限を 7.0 mg/dL とし、この値を超えた被験者については疾病罹患者と判断した。一方、血中尿酸値の平均値についてはいくつか報告があり、厚生労働省による平成 27 年国民健康・栄養調査報告においては全体の平均が 5.9 mg/dL であり、年代別での平均は 20～29 歳の 6.1 mg/dL で最も高く、75 歳以上が 5.7 mg/dL で最も低かった[2)]。その他の報告においても男性の血中尿酸値の平均値について 5.7±1.2 mg/dL[3)]としたり、年代別に集計した結果が 5.6 mg/dL（70 代以上）～6.1 mg/dL（40 代）[4)]と記載されている。以上のことから、平均値より高い値を「高め」と判断することは合理的であり、5.5 mg/dL 超～7.0 mg/dL 未満の血中尿酸値を正常値範囲の中で高めの尿酸値と設定することは妥当であると判断した。

＜本製品を用いた臨床試験の査読情報および利益相反における補足＞

1. 査読情報について

本製品を用いた臨床試験について掲載した論文は、*Integrative Molecular Medicine* に掲載されている。このジャーナルの査読については、同ジャーナルの投稿規定に下記のように記載されている。

・投稿された論文はまずセクションエディターまたは編集委員会のメンバーに割り当てられる。
・エディターが論文を査読するが、この際エディターはいかなる論文でも出版規定あるいはジャーナルの取り扱う分野、範囲から外れていた場合、その論文をリジェクトする権利を持つ。
・エディターが査読した論文は、エディターのコメントを基に外部の査読レビュワーに送られ、通常は 2～4 名の専門的な査読レビュワーに送られる。査読レビュワーはいかなる論文であっても公平かつ先入観のない査読を行う。
・最終的に査読結果は、①論文内容について比較的軽微な修正によって受理可能、②論文内容の重要な部分に関わる箇所についてある程度の修正によって受理可能、③現時点で受理はできないが更なる研究によっては受理の可能性がある、④受理できない、の 4 つに分けられる。

また、同ジャーナルは標準査読時間を暦日として 21 日と公開している。
なお、本製品を使用した臨床試験論文は 2017 年 3 月 10 日にジャーナルに受け付けられ、2017 年 3 月 27 日に受理されている。

以上のことから、本製品を使用した臨床試験について掲載した論文は、透明性の高い査読を受けて受理されたものであると判断できる。

2. 利益相反について

本製品を用いた臨床試験は、研究の発案、試験運営および資金について、いずれもオリザ油化株式会社（愛知県一宮市）が実施しており、その他の共同スポンサーはいない。論文中には著者の全てが同企業に所属していると記載されている。

＜臨床論文中のアルコールに関する記載について＞

本製品を用いた臨床試験に関する論文について、ビールおよびアルコールについての記載がある。これは日本人の尿酸値に対する考え方として、ビール等のアルコール飲料の摂取による尿酸値の増加を気にする傾向にあるという背景を述べているに留まり、本製品の摂取によってアルコールの過剰摂取をしてもよいという旨の記載ではない。

【参考文献】

1) 高尿酸血症・痛風の治療ガイドライン第 2 版，日本痛風・核酸代謝学会
2) 平成 27 年国民健康・栄養調査報告，厚生労働省
3) 金子希代子，痛風予防のＡ・Ｂ・Ｃ，情報計算化学生物学会 2017 年度大会
4) 梶川貴子ら，鳥取県の尿酸値の現状について，公益財団法人 鳥取県保健事業団

別紙様式（Ⅴ）－３【添付ファイル用】

＜「食事のプリン体が気になる方に」と表示する根拠について＞

尿酸は、ヒトの体内においてはプリン代謝と呼ばれる代謝経路の最終生成物である。食品中に含まれるプリン体は体内に摂取されるとプリン代謝経路によって尿酸に変換される。事実、プリン体を摂取した後の血中尿酸値を測定した臨床試験では、プリン体摂取によって血中尿酸値が大きく増加している [1, 2]。本製品を用いた臨床試験（単回摂取試験）においても、プリン体摂取後の被験者の血中尿酸値は摂取 4 時間後まで高値を示した。また、痛風治療ガイドラインでは、高尿酸血症および痛風患者の食事療法として、プリン体の 1 日摂取量が 400 mg を超えないように指導する旨が記載されている [3]。以上のことから、血中尿酸値が増加する原因にプリン体摂取が関係していると判断して差し支えないと思われる。さらに、痛風のイメージ調査では「痛い」、「贅沢な食事をする人がなる」に次いで「プリン体の摂取が多い人がなる」という声が多く [4]、プリン体ゼロをアピールした飲料が市場に定着していることからも、消費者がプリン体の摂取を気にするようになってきていると考えられる。よって、高めの尿酸値低下機能を表示しようとする本製品において、「食事のプリン体が気になる方に」と表示することは妥当であると判断した。

【参考文献】

1) Clifford A. J. et al., *Journal of Nutrition*, 106 (3), 428-434 (1976).
2) Ueda H. et al., *NutraFoods*, 14, 151-158 (2015)
3) 高尿酸血症・痛風の治療ガイドライン第 2 版，日本痛風・核酸代謝学会
4) 山中寿ら，女性，若年層にける痛風；最近の傾向；高尿酸血症と痛風，2, 23-29 (1994)

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Luteolin-rich chrysanthemum flower extract suppresses baseline serum uric acid in Japanese subjects with mild hyperuricemia

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Abstract

Background: Luteolin is a flavonoid found in various edible plants that exhibits diverse health benefits, including anti-inflammatory and anti-gout effects. However, there has been little clinical investigation of luteolin from the viewpoint of gout prevention. We conducted a clinical trial of supplementation with chrysanthemum flower extract rich in luteolin (LCE) to assess the effect on serum uric acid levels in Japanese men.

Methods: We examined the effect of LCE containing 10% luteolin in two double-blind placebo-controlled studies. In the single-dose study, fasting subjects took a capsule containing 100 mg of LCE (10 mg of luteolin) or placebo before ingestion of a high purine base test meal and the serum uric acid level was determined over time. For the repeated-administration study, the subjects ingested placebo or LCE capsule for 4 weeks. Fasting serum uric acid was evaluated before and after ingestion.

Results: Oral intake of LCE had no significant influence on serum uric acid. After 4 weeks of LCE ingestion, serum uric acid tended to decrease in the LCE group. A significant decrease of serum uric acid was observed after LCE ingestion in the subjects with a baseline uric acid level of 5.5 to 7.0 mg/dL. There were no abnormalities suggesting adverse effects during or after ingestion of LCE.

Conclusion: Ingestion of LCE for 4 weeks reduced the serum uric acid level. Luteolin may be able to prevent gout by controlling uric acid.

Abbreviations: XOD: xanthine oxidase; LCE: luteolin-rich chrysanthemum flower extract; CRP: C-reactive protein; AUC: area under the concentration vs. time curve; CFO: chrysanthemum flower oil

Backgrounds

Gout is a disorder in which accumulation of needle-shaped uric acid crystals in peripheral tissues due to hyperuricemia [1]. Nonsteroidal anti-inflammatory drugs [2], steroids [3], and colchicine [4] are prescribed to suppress acute pain and inflammation, while xanthine oxidase (XOD) inhibitors such as allopurinol are used for reduction of high blood uric acid levels [5]. Several Japanese foods have a high content of purine bases, such as nori (seaweed), shiitake (mushrooms), chicken liver, and sakura shrimp [6]. However, Japanese people tend to be more conscious about the purine base content of beer and alcoholic beverages, in spite of it being lower than in the foods mentioned above [7]. Approximately 30% of Japanese men have hyperuricemia and 0.8% of outpatients are on treatment for gout [8].

Dried chrysanthemum flowers are used as a traditional remedy for gout in China [9]. Several constituents of the flowers exhibit anti-inflammatory activity, including triterpene alcohol [10,11], octulosonic acid derivatives [12], and flavonoids [13]. Moreover, an oil containing 1% chrysanthemum flower extract polyphenol has been reported to suppress dietary hyperuricemia in rats [14]. Luteolin is a flavonoid with 4 hydroxyl groups and is found in a wide variety of edible plants, including chrysanthemum flowers [15]. It has been reported to exhibit diverse biological activities. In relation to gout, luteolin competitively inhibits XOD activity with a micromolar IC50 value [16-18]. Its inhibitory activity against XOD has been confirmed in mice after oral ingestion. In addition, luteolin suppressed enhancement of hepatic XOD activity enhanced by oxonate which induces hyperuricemia [19, 20]. Moreover, luteolin suppresses nitric oxide production by uric acid in pancreatic β-cells, which exacerbates diabetic complications [21]. However, there is only one clinical report about the influence of luteolin on the blood uric acid level [22]. Therefore, we investigated the effects of luteolin-rich chrysanthemum flower extract (LCE) in Japanese men with slightly high uric acid levels. In the first study, we investigated whether a single oral dose of LCE suppressed the postprandial increase of uric acid in subjects ingesting a diet with a high purine base content. We also investigated whether repeated ingestion of LCE could suppress the serum uric acid level.

Materials and methods

Subjects

For the single-dose study, 39 male employees of Oryza Oil & Fat Chemical Co. Ltd. aged 22 to 71 years were recruited. After receiving

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Key words: *luteolin, chrysanthemum, gout, uric acid, purine base*

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a full explanation of the study purpose and protocol, the candidate subjects agreed to a screening test, which involved intake of a high purine base test meal. Disodium 5'-guanylate (1.62 g, Cheil Jedang Indonesia PT) and sodium 5'-isosiate (1.63 g, Ajinomoto, Japan) were mixed with commercially available powdered corn soup (17.6 g, Knoll cup soup, Ajinomoto, Japan). Then 180 mL of hot water was added to dissolve the mixture and it was used as the high purine base test meal. The candidate subjects fasted from 10:00 pm on the night before the test and a blood sample was collected on the morning of the test. Then they ingested the test meal and blood samples were collected after 60 min, 120 min, and 240 min. Subjects were enrolled if the fasting serum uric acid level was 5.5 to 8.0 mg/dL and an increase of serum uric acid after ingestion of the test meal was confirmed. There were 20 subjects selected and they were aged from 22 to 70 years old.

For the repeated-administration study of LCE, healthy men aged 20 years or older were recruited from among the employees of Oryza Oil & Fat Chemical Co. Ltd. who freely gave consent to the study. Among them, 39 men aged 22 to 71 (42.8±13.3) years who did not meet any of the "exclusion criteria" below were selected as candidates. After confirmation of informed consent, fasting blood was collected to measure the baseline serum uric acid value, and then 30 participants were selected in order from the highest to lowest uric acid level. The subjects were grouped so that the average age was similar in each group. Uric acid levels ranged from 4.5 to 7.8 mg/dL (6.0±0.9 mg/dL). Exclusion criteria were as follows.

1) Current medication for any chronic symptom.

2) Severe allergy to foods or medicines.

3) Current treatment for hyperuricemia or gout.

Preparation and allocation of test samples

LCE was obtained by aqueous ethanol extraction from the flowers of *Chrysanthemum indicum* cultivated in China and powdered by Oryza Oil & Fat Chemical Co. Ltd. LCE (commercial name: Kiku Flower Extract-P; Lot. T-519) contained 10% luteolin, and 100 mg of LCE was placed into colored hard capsules for ingestion. Placebo capsules with the same appearance as the LCE capsules that contained 100 mg of dextrin were also made. The capsules were colored and the filling was not visible from outside. The allocation sheet indicating test sample allocation was strictly protected by a controller who was not directly involved in the study.

For the single-dose study, the 20 subjects were allocated to 2 groups so that the average age and uric acid level were similar. For the repeated-administration study, the 30 subjects were allocated to 2 groups from the highest value to lowest uric acid value.

Protocol

The studies and statistical analysis were carried out at Oryza Oil & Fat Chemical Co. Ltd. The studies were done as placebo-controlled double-blind cross-over trials. The primary outcome was the serum uric acid level in both studies.

For the single-dose study (performed on July 8, 2016), one group (n=10) ingested a placebo capsule and the other group ingested an LCE capsule (n=10). All subjects fasted from 10:00 pm on the night before the test and the first blood collection was carried out in the morning of the test day. Then the subjects ingested the high purine base test meal and the second, third, and fourth blood collections were performed after 60, 120, 180 and 240 min. Urine was collected 2 hr after intake of the test meal. At seven days after the first examination, the same test was repeated. The subjects in each group ingested the other type of capsule and the test meal, and blood was collected in a similar way to that described above.

For the repeated-administration study, blood and urine were collected from fasting subjects in both groups on September 6, 2016. The subjects in each group (N=15) ingested one placebo capsule or one LCE capsule daily with water after a meal for 4 weeks. Then blood and urine samples were collected from fasting subjects. After a 13-day washout period, blood and urine samples were collected from the fasting subjects again. Then the subjects in each group ingested the other type of capsule (LCE or placebo) for 4 weeks, after which blood and urine samples were collected under fasting conditions.

Blood and urine analysis

In the single-dose study, the collected blood samples were centrifuged to obtain serum. Uric acid levels in serum and urine were determined by FALCO Biosystems Ltd. (Kyoto, Japan).

In the repeated-administration study, serum was obtained for determination of uric acid and C-reactive protein (CRP), while whole blood was used for measurement of the erythrocyte sedimentation rate and white blood cell count. These parameters were determined by FALCO Biosystems Ltd. Urine pH was measured by pH papers ranged from 3.8 to 5.4 and 5.5 to 9.0.

Ethics and compliance

This study was performed in accord with the WMA Declaration of Helsinki (64th WMA General Assembly in 2013, Brazil) and was carried out in line with ethical considerations. Each subject's human rights and safety were taken into consideration. The ethics committee of Oryza Oil & Fat Chemical was made up of scientists from Oryza Oil & Fat Chemical Co. Ltd. with doctoral degrees in pharmacy, as well as a medical doctor and a lawyer from a legal firm. The committee was convened to assess the ethical nature and appropriateness of the protocol. The two studies were implemented based on the protocol approved by the ethics committee and any substantial deviations from the protocol required authorization by the committee. Both studies were registered with the University Hospital Medical Information Network-Clinical Trial Registry (UMIN000023818 and UMIN000022823).

Investigation of adverse events

Evaluation of adverse events was carried out and the causal relationship with the test substances was determined. The decision as to whether or not the study should continue was made by the doctor in charge, if necessary. Adverse events were defined as symptoms resulting from ingestion of LCE that were unpleasant for the subject. If a subject asked to discontinue the study, it was promptly stopped and consideration was taken to prevent any disadvantage to the subject.

Exclusion criteria

If any of the following events occurred during the repeated-administration study, the case was discussed at the clinical conference and the subject in question was excluded from analysis after review.

1) If the subject was late when participating in each test.

2) If the number of days with no intake of the test product (i.e., the specified daily amount for ingestion was not reached) was greater than 15 % of the total number of planned ingestion days.

3) If there were major issues with regard to reliability of the data due to problems with testing or other reasons.

4) Other reasons suggesting that it was appropriate to exclude the subject.

Statistical analysis

The results are reported as the mean and SD (except urine parameters). A two-tailed paired *t*-test was used for comparisons between before and after ingestion of capsules. The unpaired *t*-test was used to compare the placebo group with the LCE groups. A probability of less than 5% was considered significant.

Results

Single-dose study

The results of the tests performed before and after the washout period were combined (20 subjects in total). The serum uric acid level before ingestion of the placebo or LCE capsules was 6.8±1.0 mg/dL and 6.6±1.0 mg/dL, respectively. At 2 hr after the test meal ingestion, serum uric acid rose to 9.1±1.3 mg/dL (placebo) and 9.0±1.3 mg/dL (LCE). Uric acid remained at more than 8.5 mg/dL at 4 hr after the test meal. The area under the concentration vs. time curve (AUC) was almost the same in both groups, being 457±179 mg/dL·4 hr in the placebo group and 469±201 mg/dL·4 hr in the LCE group. Figure 1A shows the increase of serum uric acid in all subjects and in subjects with a uric acid level of less than 7.0 mg/dL. There were no significant differences up to 4 hr after the test meal. Urinary excretion of uric acid was similar in both groups (Figure 1B).

Four-week repeated-administration study

Two subjects dropped out from each of the placebo and LCE groups during the study period for personal reasons. Table 1 shows the changes of serum uric acid after ingestion of placebo capsules or LSE capsules. Ingestion of LSE caused uric acid to decrease significantly (-0.18 mg/dL). Statistical analysis was also performed in subjects with a uric acid level of more than 5.5 and less than 7.0 mg/dL. As a result, significant reduction of the uric acid level was observed in LCE group compared to the placebo group. The changes of blood pressure, pulse rate, and urine pH (safety evaluation parameters) are indicated in Table 2. All values were in the normal ranges and significant differences were not detected between before and after ingestion or between the two groups. Moreover, inflammatory parameters (CRP, erythrocyte sedimentation rate, and white blood cell count) did not change during ingestion of placebo capsules or LCE capsules (Table 3).

Table 1. Serum uric acid level before and after ingestion of placebo or LCE for 4 weeks.

		Before	After	Net change (Δ)
First period				
Group A	Placebo	5.84 ± 0.94	5.88 ± 0.94	0.05 ± 0.55
Group B	LCE	6.12 ± 1.10	5.98 ± 1.07	-0.14 ± 0.37
Second period				
Group A	LCE	6.08 ± 1.27	5.82 ± 1.02	-0.26 ± 0.50
Group B	Placebo	5.88 ± 0.93	5.65 ± 0.88†	-0.23 ± 0.38
Total (All subjects)				
Placebo		5.96 ± 1.10	5.85 ± 0.96	-0.11 ± 0.54
LCE		6.00 ± 1.00	5.82 ± 0.97†	-0.18 ± 0.37
Total (uric acid of 5.5 to 7.0 mg/dL)				
Placebo		5.91 ± 0.59	6.09 ± 0.52	0.18 ± 0.48
LCE		6.18 ± 0.65	5.98 ± 0.55	-0.20 ± 0.38*

Values represent the mean and SD (n=13 or 26). Significant differences between placebo and LCE*: $p<0.05$. Significant difference between before and after ingestion †: $P < 0.05$.

Table 2. Blood pressure, pulse rate, and urine pH before and after ingestion of LCE or placebo.

		Before			After 4 weeks			Net change (Δ)		
Systolic blood pressure (mmHg)	Placebo	136.2	±	13.1	132.8	±	15.8	-3.4	±	14.5
	LCE	134.5	±	13.7	130.5	±	15.7	-3.9	±	10.5
Diastolic blood pressure (mmHg)	Placebo	81.5	±	8.6	79.6	±	8.9	-1.9	±	8.1
	LCE	80.4	±	11.7	79.2	±	10.0	-1.3	±	9.9
Pulse rate (/ min)	Placebo	78.1	±	17.9	77.8	±	13.3	-0.3	±	10.5
	CFE	73.8	±	14.2	79.2	±	10.0	5.4	±	14.5
Urine pH	Placebo	6.63	±	0.48	6.67	±	0.49	0.04	±	0.47
	CFE	6.52	±	0.54	6.69	±	0.49	0.17	±	0.45

Values represent the mean and SD (n=26). No significant differences were detected between before and after ingestion or between the two groups.

Discussion

We investigated the effect of LCE on the serum uric acid level in healthy Japanese subjects. It was found that a single oral dose of LCE did not suppress the increase of uric acid after a high purine base test meal. Ueda *et al*. reported that chrysanthemum flower oil (CFO) containing luteolin suppressed elevation of serum uric acid in Japanese subjects after loading with a high purine base diet [22]. In their study, significant suppression of uric acid by CFO was observed in the subjects with a baseline serum uric acid level of 7.1 mg/dL or more, while there was no significant difference between the placebo and CFO groups in subjects with a baseline serum uric acid of 6.5 mg/dL or more. In Japan, the criterion for diagnosis of hyperuricemia is a serum uric acid level of more than 7.0 mg/dL. In this study, we examined the effect of LCE on Japanese men with serum uric acid levels over the range from normal to hyperuricemia (5.5 to 8.0 mg/dL). However, LCE did not suppress the increase of uric acid in all subjects or in the subjects with uric acid levels of less or more than 7.0 mg/dL (data not shown). Hence, CFO seems to reduce the postprandial uric acid level in patients with hyperuricemia, but LCE has no effect on postprandial elevation of uric acid. Differences in the constituents of CFO and LCE may have contributed to the differing results. The flavonoid content of CFO was not mentioned in the report, but the luteolin content of LCE is 10% and the content of other polyphenolic compounds is relatively lower. Thus, luteolin (a principal component of LCE) does not seem to affect to postprandial elevation of the serum uric acid level.

After ingestion of LCE for 1 month in the second study, the serum uric acid level decreased in the subjects with a baseline uric acid level of 5.5 to 7.0 mg/dL. Hence, LCE seems to suppress mildly elevated uric acid levels. Among structurally similar flavonoid derivatives, consumption of quercetin (500 mg/day) for 4 weeks has been reported to decrease the baseline serum uric acid level without affecting uric acid excretion [23]. In addition, 6-month consumption of soy bean-derived daizein (40 mg/day) decreased serum uric acid in hypercholesterolemic subjects [24] Among processed foods containing polyphenols, green tea [25] and black tea [26] extracts have been reported to suppress the serum uric acid level. Therefore, there is evidence that polyphenolic compounds like flavonoids and catechin derivatives may reduce the baseline serum uric acid level. Thus, luteolin in LCE might also have suppressed baseline serum uric acid by a similar mechanism to that reported for polyphenols.

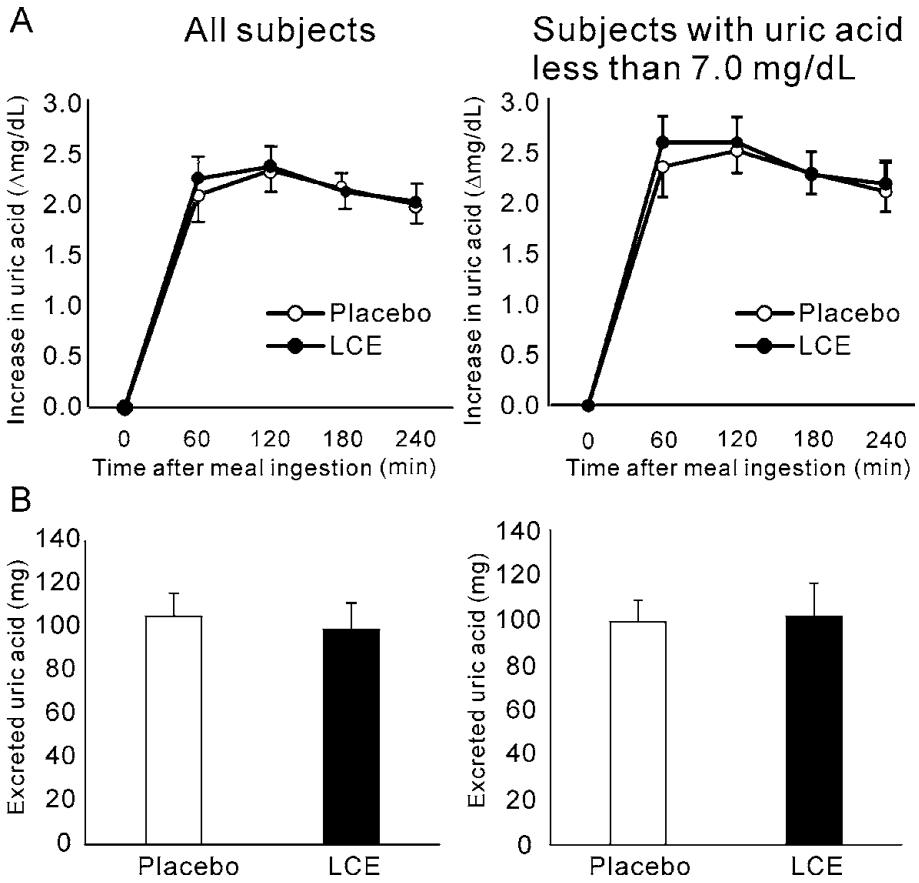
Conclusion

Ingestion of LCE for 4 weeks suppressed the fasting serum uric acid level in Japanese men with mild hyperuricemia.

Table 3. Laboratory parameters before and after ingestion of LCE or placebo.

Parameter	Unit	Placebo									LCE								
		Before			After			Net change (Δ)			Before			After			Net change (Δ)		
C-reactive protein	mg / dL	0.17	±	0.35	0.09	±	0.08	-0.08	±	0.33	0.10	±	0.15	0.12	±	0.02	0.01	±	0.13
Erythrocyte sedimentation rate (1 hour)	mm	4.7	±	4.2	4.9	±	4.6	0.2	±	3.8	4.4	±	4.1	4.6	±	0.8	0.2	±	1.4
Erythrocyte sedimentation rate (2 hours)	mm	12.4	±	11.1	13.2	±	11.8	0.8	±	8.9	11.0	±	10.4	12.4	±	1.9	1.4	±	3.5
White blood cell count	$\times 10^2$ / μL	61.5	±	14.4	56.0	±	12.7	-5.5	±	14.6	59.1	±	15.3	59.6	±	2.7	0.5	±	18.7

Values represent the mean and SD (n=26). No significant differences were detected between before and after ingestion or between the two groups.



Values represent the mean and SD (n=20). There were no significant differences between the LCE and placebo groups.

Figure 1. Change of serum uric acid after a single oral dose of LCE in subjects given a high purine base test meal.

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Conflict of interest

All authors related to this study are employees of Oryza Oil & Fat Chemical Co., Ltd. (Aichi, Japan). The authors declare no conflict of interest associated with this manuscript.

References

1. Dalbeth N, Merriman TR, Stamp LK (2016) Gout. *Lancet* 388: 2039-2052. [Crossref]
2. Janssens HJ, Janssen M, van de Lisdonk EH, van Riel PL, van Weel C (2008) Use of oral prednisolone or naproxen for the treatment of gout arthritis: a double-blind, randomised equivalence trial. *Lancet* 371: 1854-1860. [Crossref]
3. Rainer TH, Cheng CH, Janssens HJ, Man CY, et al. (2016) Oral prednisolone in the treatment of acute gout: a pragmatic, multicenter, double-blind, randomized trial. *Ann Intern Med* 164: 464-471. [Crossref]
4. Wang Y, Wang L, Li E, Li Y, Wang Z, et al. (2014) Chuanhu anti-gout mixture versus colchicine for acute gouty arthritis: a randomized, double-blind, double-dummy, non-inferiority trial. *Int J Med Sci* 11: 880-885. [Crossref]
5. Takir M, Kostek O, Ozkok A, Elcioglu OC, Bakan A, et al. (2015) Lowering uric acid with allopurinol improves insulin resistance and systemic inflammation in asymptomatic hyperuricemia. *J Investig Med* 63: 924-929.
6. Kaneko K, Aoyagi Y, Fukuuchi T, Inazawa K, Yamaoka N (2014) Total purine and purine base content of common foodstuffs for facilitating nutritional therapy for gout and hyperuricemia. *Biol Pharm Bull* 37: 709-721
7. Inokuchi T, Ka T, Yamamoto A, Takahashi S, Tsutsumi Z, et al. (2008) Effects of allopurinol on beer-induced increases in plasma concentrations of purine bases and uridine. *Nucleosides Nucleotides Nucleic Acids* 27: 601-603. [Crossref]
8. Hakoda M (2012) Recent trends in hyperuricemia and gout in Japan. *Japan Med Assoc J* 55: 319-323. [Crossref]
9. Kong LD, Cai Y, Huang WW, Cheng CH, Tan RX (2000) Inhibition of xanthine oxidase by some Chinese medicinal plants used to treat gout. *J Ethnopharmacol* 73: 199-207. [Crossref]
10. Akihisa T, Yasukawa K, Oinuma H, Kasahara Y, Yamanouchi S, et al. (1996) Triterpene alcohols from the flowers of compositae and their anti-inflammatory effects. *Phytochemistry* 43: 1255-1260. [Crossref]
11. Ukiya M, Akihisa T, Yasukawa K, Kasahara Y, Kimura Y, et al. (2001) Constituents of compositae plants. 2. Triterpene diols, triols, and their 3-*O*-fatty acid esters from edible chrysanthemum flower extract and their anti-inflammatory effects. *J Agric Food Chem* 49: 3187-3197. [Crossref]

12. Luyen BT, Tai BH, Thao NP, Cha JY, Lee HY, et al. (2015) Anti-inflammatory components of *Chrysanthemum indicum* flowers. *Bioorg Med Chem Lett* 25: 266-269. [Crossref]

13. Chen XY, Li J, Cheng WM, Jiang H, Xie XF, et al. (2008) Effect of total flavonoids of *Chrysanthemum indicum* on the apoptosis of synoviocytes in joint of adjuvant arthritis rats. *Am J Chin Med* 36: 695-704. [Crossref]

14. Honda S, Kawamoto S, Tanaka H, Kishida H, Kitagawa M, et al. (2014) Administered chrysanthemum flower oil attenuates hyperuricemia: mechanism of action as revealed by DNA microarray analysis. *Biosci Biotechnol Biochem* 78: 655-661. [Crossref]

15. Chen L, Kotani A, Kusu F, Wang Z, Zhu J, et al. (2015) Quantitative comparison of caffeoylquinic acids and flavonoids in *Chrysanthemum morifolium* flowers and their sulfur-fumigated products by three-channel liquid chromatography with electrochemical detection. *Chem Pharm Bull (Tokyo)* 63: 25-32. [Crossref]

16. Yan J, Zhang G, Hu Y, Ma Y (2013) Effect of luteolin on xanthine oxidase: inhibition kinetics and interaction mechanism merging with docking simulation. *Food Chem* 141: 3766-3773. [Crossref]

17. Li Y, Frenz CM, Li Z, Chen M, Wang Y, et al. (2013) Virtual and *in vitro* bioassay screening of phytochemical inhibitors from flavonoids and isoflavones against xanthine oxidase and cyclooxygenase-2 for gout treatment. *Chem Biol Drug Des* 81: 537-544. [Crossref]

18. Kong LD, Wolfender JL, Cheng CH, Hostettmann K, Tan RX (1999) Xanthine oxidase inhibitors from *Brandisia hancei*. *Planta Med* 65: 744-746. [Crossref]

19. de Souza MR, de Paula CA, Pereira de Resende ML, Grabe-Guimarães A, de Souza Filho JD, et al. (2012) Pharmacological basis for use of *Lychnophora trichocarpha* in gouty arthritis: anti-hyperuricemic and anti-inflammatory effects of its extract, fraction and constituents. *J Ethnopharmacol* 142: 845-850. [Crossref]

20. Mo SF, Zhou F, Lv YZ, Hu QH, Zhang DM, et al. (2007) Hypouricemic action of selected flavonoids in mice: structure-activity relationships. *Biol Pharm Bull* 30: 1551-1556. [Crossref]

21. Ding Y, Shi X, Shuai X, Xu Y, Liu Y, et al. (2014) Luteolin prevents uric acid-induced pancreatic β-cell dysfunction. *J Biomed Res* 28: 292-298. [Crossref]

22. Ueda T, Honda S, Morikawa H, Kitamura S, Iwama Y, et al. (2015) Chrysanthemum flower oil inhibits diet-induced serum uric acid elevation in adult male subjects. A randomized, double-blind, placebo-controlled cross-over comparison study. *Nutrafoods* 14: 151-158.

23. Shi Y, Williamson G (2016) Quercetin lowers plasma uric acid in pre-hyperuricaemic males: a randomized, double-blinded, placebo-controlled, cross-over trial. *Br J Nutr* 115(5): 800-6. [Crossref]

24. Kimira M, Arai Y, Shimoi K, Watanabe S (1998) Japanese intake of flavonoids and isoflavonoids from foods. *J Epidemiol* 8: 168-175. [Crossref]

25. Jatuworapruk K, Srichairatanakool S, Ounjaijean S, Kasitanon N, Wangkaew S, et al. (2014) Effects of green tea extract on serum uric acid and urate clearance in healthy individuals. *J Clin Rheumatol* 20: 310-313. [Crossref]

26. Bahorun T, Luximon-Ramma A, Gunness TK, Sookar D, Bhoyroo S, et al. (2010) Black tea reduces uric acid and C-reactive protein levels in humans susceptible to cardiovascular diseases. *Toxicology* 278: 68-74. [Crossref]