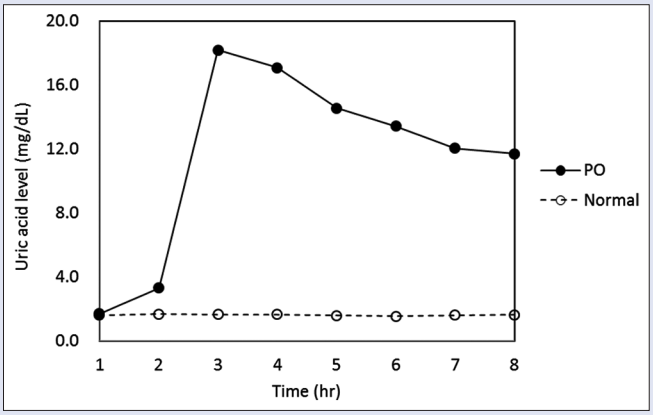

Figure 2: Time-course effect of potassium oxonate on hyperuricemic mice uric acid levels

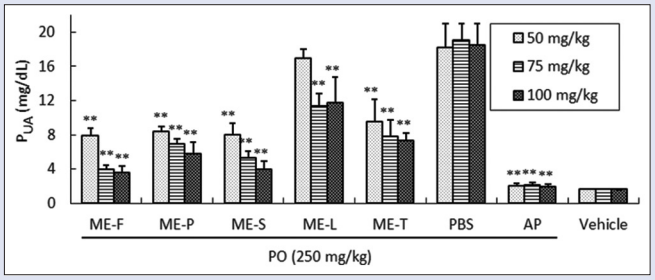

Figure 3: The uric acid-lowering effects of methanol extract from longan flower, pericarp, seed, leaf and twig tissues (50, 75, 100 mg/kg) on mice with PO-induced hyperuricemia. The results are presented as the mean ± SD ($n = 6$). $^{**}P < 0.01$ compared to the PO-treated group

Determination of xanthine oxidase-inhibitory activity

XO-inhibitory activity was measured spectrophotometrically based on the procedure reported by Nguyen *et al.*[26] The reaction mixture consisted of 50 µL of test samples or compounds, 35 µL of 50 mM phosphate buffer (pH 7.5), and 30 µL of XO solution (0.1 U/mL in 50 mM phosphate buffer, pH 7.5) and was prepared immediately before use. After preincubation at RT (25°C) for 15 min, 60 µL of substrate solution (150 µM xanthine in the same buffer) was added to the mixture to initiate the reaction. The assay mixture was then incubated at RT for 30 min. Afterward, 25 µL of stop solution (1 N HCl) was added, and the absorbance values were measured at 290 nm with a microplate reader (µQuant™, BIO-TEK Instruments Inc., USA). Allopurinol was used as a positive control. Three replicates were performed for each test sample, and the increased ultraviolet absorption at 290 nm indicated the formation of uric acid. The percentage inhibition ratio was calculated according to the following equation: % inhibition = (1 − B/A) × 100, where A is the change in absorbance per min without the test sample and B is the change in absorbance per min with the test material. The concentration of samples required to inhibit 50% of XO activity (IC_{50}) was estimated from the % inhibition versus concentration plot using a linear regression algorithm.[27]

Hyperuricemia model in mice

About 6–8-week-old male ICR mice weighing 25–30 g were purchased from BioLASCO Taiwan Co., Ltd. (Yilan, Taiwan), maintained in 12 h light/dark cycles, and housed at 23 ± 2°C for at least 1 week prior to the experiment. Animals were provided with a rodent diet and clean water *ad libitum*, except 1 h prior to drug administration when access to food was restricted. Animal tests used in this study were conducted under the guidelines of the International Association for the Study of Pain.[28] The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of National Taiwan University (IACUC Approval No: NTU-101-EL-14).

Experimental hyperuricemia was induced in mice by intraperitoneal (i.p.) injections of the uricase inhibitor PO as described previously.[29] The mice were i.p. injected with phosphate-buffered saline (PBS) containing PO (250 mg/kg) 1 h after administration of test samples to increase blood urate levels. The names of the samples are as follows: Methanol extracts of the flower (F), pericarp (P), seed (S), leaf (L), and twig (T) of longan were shortened for ME-F, ME-P, ME-S, ME-L, and ME-T, respectively. Mice were randomly assigned into the following seven groups for different treatments ($n = 6$): (1) Vehicle group (normal control); (2) PO + allopurinol (AP, 10 mg/kg) group (positive control); (3) PO + PBS group (negative); (3) PO + ME-F group; (4) PO + ME-P group; (5) PO + ME-S group; (6) PO + ME-L group; (7) PO + ME-T group. For the comparative study, three dosages (100, 75, and 50 mg/kg) were delivered to ensure the utilization of this extract.

Measurement of plasma uric acid level

Uric acid in tail vein blood was measured 2 h after PO injection using commercial Ektachem clinical chemistry slides from Johnson & Johnson clinical diagnostics (US).

Statistical analysis

All data were expressed as the mean ± standard deviation ($n = 3$). The significance of difference was performed with Duncan's new multiple range test, and $^{*}P < 0.05$ and $^{**}P < 0.01$ were considered statistically significant.

RESULTS

Time course potassium-oxonate effects on plasma uric acid levels in mice

The effects of PO on mice plasma uric acid (P_{UA}) levels are shown in Figure 2. Uric acid levels in nonhyperuricemic, vehicle-treated mice were 1.6 ± 0.05 mg/dL. However, treatment with uricase inhibitor PO resulted in a significant elevation of P_{UA} levels reaching to 18 ± 2.8 mg/dL after 2 h, followed by slow decrease in urate levels 8 h postinjection [Figure 2].

Xanthine oxidase-inhibitory activity of longan crude extracts and derived soluble fractions

The tested longan crude extracts inhibited XO in a concentration-dependent manner. Longan flowers showed the best XO-inhibitory activity, with an IC_{50} value of 115.8 µg/mL, followed by pericarps (118.9 µg/mL), twigs (125.3 µg/mL), seeds (262.5 µg/mL), and leaves (331.1 µg/mL), respectively. These results are in accordance with Hou *et al.*,[21] who reported that longan seed extract had dose-dependent XO-inhibitory activity with an IC_{50} value of 277.8 µg/mL. Comparisons of XO-inhibitory activity results [Table 2] in various derived soluble fractions from longan extracts indicated that there are abundant XO-inhibitory phytochemicals present in longan extracts, especially in the EA fraction [Table 2].

Xanthine oxidase-inhibitory activity of phytochemicals from longan flower extracts

The inhibitory effects of isolated 10 phytochemicals [Figure 1] from longan flower extracts against XO are given in Table 3. The experimental evidence indicates that proanthocyanidin A2 and acetonylgeraniin A showed an excellent activities' profile for inhibition to XO compared to that of the standard, allopurinol, as indicated by inhibition (%). In addition, proanthocyanidin A2, a phenolic dimer belonging to the class of condensed tannins, has been shown to display superior antioxidant activity to that of ascorbic acid in previous research works;[6,30] while some studies revealed that acetonylgeraniin A, a hydrolyzable tannin, has an antihypertensive effect.[31,32] In this study, proanthocyanidin A2 and acetonylgeraniin A were found to inhibit XO *in vitro*, which may be potentially useful for the treatment of gout [Table 3].

In vivo hypouricemic effect determined in mice with potassium-oxonate-induced hyperuricemia

To further confirm the capabilities of methanol extract of longan flower, pericarp, seed, leaf, and twig tissues to reduce the uric acid level *in vivo*, a PO-induced hyperuricemia mice model was investigated. In vehicle control mice, the P_{UA} level was 1.6 ± 0.03 mg/dL. After 2 h of PO treatment, the level of P_{UA} had increased to 18.5 ± 0.4 mg/dL. As shown in Figure 3, oral administration of ME-F, ME-P, ME-S, ME-L, and ME-T (50, 75 and 100 mg/kg) significantly reduced plasma urate levels in hyperuricemic mice in a dose-dependent manner, as well as the reference (AP) group. The administration of allopurinol (PO + AP group) significantly reduced the level of P_{UA} by (2.0 ± 0.1 mg/dL) 89% as compared with the PO + PBS group ($P < 0.01$). At doses of 75 mg/kg of ME-F, ME-P, ME-S, ME-L, ME-T, or above, plasma urate levels of the PO-treated mice were significantly reduced by 80%, 64%, 72%, 41%, and 59%, respectively, relative to the PO + PBS group ($P < 0.01$). No significant difference existed between the dosages of longan extracts at 75 and 100 mg/kg. Conversely, the lowering effect on uric acid by longan extracts at 50 mg/kg on PO-induced hyperuricemic mice was found to be weaker than that observed for 75 and 100 mg/kg dosages. It is noteworthy that comparisons of these results indicate that ME-F and ME-S exhibit excellent hypouricemic effects. Remarkably, the methanol extract of the flowers was more potent than seeds in uric acid-lowering effects *in vivo* [Figure 3].

DISCUSSION

In recent years, there is an increasing interest in finding herbal plants and phytochemicals which possess the capacity to inhibit XO activity and reduce urate levels. Longan is a fruit used in herbal preparations in China, and though unpollinated longan flowers and nonedible fruit seeds are generally regarded as disposable byproducts, studies show that longan flowers, pericarps, and seeds contain high levels of phenolics and flavonoids, which exhibit high antioxidant activity and may be rendered suitable as protective agents [Table 1]. The activities of extracts from its flowers, pericarps, leafs, and twigs against XO are reported here for the first time. A study was demonstrated that longan seed extract and its active components (corilagin, gallic acid, and ellagic acid) inhibited XO dose dependently *in vitro*, but were less potent than allopurinol.[21] Our findings indicate that extracts from longan flowers have a great potential for preventing diseases caused by the XO-inhibitory activity *in vitro*, with an IC_{50} value of 115.8 μg/mL, followed by pericarps (118.9 μg/mL), twigs (125.3 μg/mL), seeds (262.5 μg/mL), and leaves (331.1 μg/mL). For the *in vivo* study, longan extract (75 mg/kg) was able to reduce P_{UA} levels and XO activities in hyperuricemic mice in a decreasing order: ME-F (80%) > ME-S (72%) > ME-P (64%) > ME-T (59%) > ME-L (41%), compared with allopurinol (89%). Meanwhile, 10 phytochemicals were identified from longan flower, and a superior XO-inhibitory activity in the type of phenolics was observed. The *in vitro* inhibitions of XO by proanthocyanidin A2 and acetonylgeraniin A are high when compared to allopurinol, which possess the hypouricemic activities for the first time. Others yield weak inhibitory activity against XO.

A toxicological study revealed no toxic effects of oral administration of longan seed extract during acute and repeated doses (4 and 13 weeks).[33] Besides, longan seed extract inhibited uric acid production and XO activity in normal liver cells (clone-9 cells) and was not cytotoxic under the concentration of 200 μg/ml.[21] The results suggested that its urate-reducing effect might be due to modulation of urate transporters (GLUT1 and GLUT9) and inhibition of circulating XO. It is of great interest that the XO-inhibitory effect of longan flowers and seeds, the byproduct materials, may provide some choices for prevention and/or treatment of hyperuricemia as valuable functional ingredients.

CONCLUSION

It can be concluded from the present finding that longan extracts possess potent *in vivo* hypouricemic effects in hyperuricemic rats pretreated with oxonate. The use of longan flowers and seeds in the treatment of gout could be attributed to its inhibitory effect on XO. These results also suggest that proanthocyanidin A2 and acetonylgeraniin A extractions from *Dimocarpus longan* Lour. flowers could be developed as potent XO inhibitors for clinical application.

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Nil.

Conflicts of interest

All authors declare that they have no conflicts of interest.

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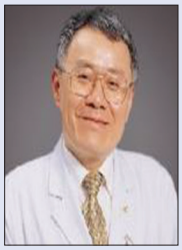

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