

ANTIHYPERURICEMIC EFFECT OF THE FREEZE-DRIED AQUEOUS EXTRACT OF *Peperomia pellucida* (L) HBK (*ulasimang bato*) IN RATS

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ABSTRACT

Objective

To determine if *Peperomia pellucida* freeze-dried aqueous extract powder can lower the serum uric acid level (SUA) of hyperuricemic rats

Study design

This was a randomized controlled study among 15 male adult Sprague Dawley rats in three different treatment groups: allopurinol 60 mg/kg body weight as positive control, *P pellucida* 1 g/kg body weight as test drug, and normal saline solution 0.9% as negative control.

Outcome measure

Mean % decrease from hyperuricemic level was used to assess the urate-lowering activity.

Results

Mean % decrease in elevated SUA three hours after treatment was in the decreasing order of allopurinol > *P pellucida* > saline, (64.0 % > 44.1 % > 10.4 %), with paired t test, alpha = 0.05.

Conclusion

The plant had a mean % decrease from hyperuricemic level of 44.1 % compared to allopurinol's 64.0 %, indicating that *P pellucida* may be used as an alternative medication for hyperuricemia.

Key words

Peperomia pellucida, antihyperuricemia, natural product

INTRODUCTION

Prolonged hyperuricemia carries a risk for gouty arthritis and renal stone formation.

The Framingham study indicated that the prevalence of gout increases with increasing serum uric acid level (1). Yu and Gutman indicated that the prevalence of renal calculus development increases with increasing serum urate concentration (2).

The lowering of elevated serum uric acid level is therefore an important step in the treatment of gouty arthritis and in the

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prevention of uric acid stone formation.

Dietary control of purine intake reduces the mean serum urate concentration by only 1.0 mg/dL and decreases the urinary uric acid excretion by 200-400 mg/day (3-5). When hyperuricemia persists inspite of dietary control, medication is indicated (6-7).

The high cost of medication, however, hinders good compliance. Moreover, although allopurinol is well tolerated by most patients, reactions to drug such as skin rash, gastrointestinal distress, diarrhea, hepatic toxicity occur in up to 20% of the treated patients. This necessitates the drug discontinuance in 5% of the patients (8).

Thus, efforts are now focused on medicinal plants like *Peperomia pellucida*, locally known as *ulasimang bato* or *pansit-pansitan*, which is being used as a more affordable and readily available alternative medication for gout, kidney trouble and rheumatic pain.

However, it was noted that the scientific validation of the efficacy of *P pellucida* is lacking and this was the focus of the study.

The general objective of the study was to determine if *P pellucida* freeze-dried aqueous extract powder can lower serum uric acid level in hyperuricemic rats. The specific objectives of the study were as follows:

1. To determine the serum uric acid levels of hyperuricemic rats treated with *P pellucida* 1 g per kg body weight (test drug), or allopurinol 60 mg per kg body weight (positive control), or 0.9% normal saline solution (negative control).
2. To compare the serum uric acid levels of hyperuricemic rats among the three treatment groups.

MATERIALS AND METHODS

Preparation of sample

The method of preparation for the test drug is described in a related article in this journal.

Test animal

Male adult Sprague Dawley rats were obtained from the Laboratory Animal House at the University of the Philippines Diliman. The animals were acclimatized to the Pharmacology laboratory, and were given water and pigeon pellets ad libitum. Fifteen animals were randomized into three treatment groups. At the time of the experiment, the rats were 8-month old weighing about 300 g.

Uric acid test

Uric acid test kit (lot 071421) was obtained from Eagle Diagnostics, USA using TPTZ (2,4,6 - tripyridyl-s-triazine) method of analysis. Perkin Elmer Coleman spectrophotometer Model 6/20 with a 10 mm round cell cuvette and a microspace adapter was used in the analysis of serum uric acid.

Control materials

Two levels of commercially available control materials, with values focused at normal and abnormal concentrations, were selected. Normal control used was Qualitrol lot 3604, with an expected range of 4.30 - 6.39 mg/dL assayed by uricase method. Abnormal control used was Monitrol II lot PTS110, with an expected range of 7.90 - 9.80 mg/dL assayed by uricase method. At least twelve determinations, carried out in seven different days, were used to monitor the precision and accuracy of the uric acid TPTZ test method (9-11). Both normal and abnormal controls were assayed alongside rat samples.

Oxonate-induced hyperuricemia

To assess the efficacy of the urate-lowering therapy, a hyperuricemic level was first established (Figure 1). About 0.3 mL blood was collected via the rat's tail to determine the baseline serum uric acid level (Day 0 Hour 0 [= baseline] SUA) (12). Immediately after, hyperuricemia was induced by an oral gavage feeding of potassium oxonate 250 mg/kg body weight and uric acid 50 mg/kg body weight (13). After 3 hours, blood was collected to confirm hyperuricemia (Day 0 Hour 3 SUA). In oral dosing, the volume of material administered did not exceed 2-3% of the body weight (14). Potassium oxonate 97% for laboratory use was purchased from Aldrich Chemical Co, lot 08122 LW and from Sigma Chemical Co, lot 52H 3629. Uric acid 98% for biochemistry grade was purchased from Merck, lot 819 K2338717.

Outcome measure. SUA levels before [=baseline] and after oxonate treatment on Day 0 were compared. Mean % increase in SUA, arbitrarily set at a minimum of 40% increase, was considered as hyperuricemic (acute hyperuricemia). Having attained prolonged hyperuricemia was evaluated by conducting daily oxonate-urate challenge and determining if the persistent elevated uric acid level was achieved.

Data analysis. Paired t test was used to check on the difference before and after oxonate treatment. One-way analysis of variance was used to check the comparability of the 3 treatment groups.

Urate-lowering activity

The following day (Day 1), after one hour of oxonate-urate challenge, oral drug treatment was given to hyperuricemic rats (Figure 1). Positive control group was given allopurinol 60 mg/kg body weight (allopurinol 100 mg/tablet, Purinase® manufactured by

United Laboratories Inc, lot 2141101, purchased from the drug store). The dose was based on the daily moderate dose of 600 mg for a 70 kg man and extrapolating this to rat using the surface area ratio (15).

$$600 \text{ mg} \times \frac{0.018}{20 \text{ g rat}} \times \frac{1000 \text{ g}}{1 \text{ kg}} = 54 \text{ mg/kg rat}$$

Test drug group was given freeze-dried aqueous extract powder of *P pellucida* 1 g/kg body weight, prepared from a 100% decoction of fresh aerial parts of the mature plant. The dose was based on $\frac{1}{10}$ of LD₅₀ of mouse (16) and extrapolating this to rat using the surface area ratio. The oral LD₅₀ of *P pellucida* in water was 11.78 g/kg body weight of mouse \pm 0.69 SE for male and female mice observed over a 14-day period as described in a related article in this journal.

$$\frac{1}{10} \times \frac{11.78 \text{ g}}{\text{kg mouse}} \times \frac{1 \text{ kg}}{1000 \text{ g}} \times 20 \text{ g mouse} \times \frac{7}{200 \text{ g rat}} \times \frac{1000 \text{ g}}{1 \text{ kg}} = 0.8239 \text{ g/kg rat}$$

Negative control group was given normal saline solution 0.9%.

After 1 hour of oxonate-urate challenge followed by 2 hours after drug treatment, blood was collected for serum uric acid determination (Day 1 Hour 3 SUA). Hour 3 SUA was the time interval selected because the analyte was highest at hour 3 based on the in-house information. The oxonate-urate challenge was given daily for 14 days prior to the daily drug treatment to simulate the persistent elevated uric acid level of gouty patients. Hour 3 SUA was monitored after 1 day (Day 1), after 1 week (Day 7) and after 2 weeks (Day 14) of daily drug treatment.

Outcome measure. Mean SUA among the three treatment groups were compared. Mean % decrease in SUA from hyperuricemic level after drug treatment was considered as urate-lowering activity of the drug.

Data analysis. One-way analysis of covariance (ANOCOVA) followed by Duncan's multiple range test was used in comparing the treatment means (17-18).

Analysis of variance (ANOVA) with repeated measure over time (Days 1,7,14) was not used since preliminary trials

revealed that only acute hyperuricemia, and not prolonged hyperuricemia, was attained. To maximize the use of the limited number of animals, SUA levels were taken but were considered as triplicates. The average was then taken. Hence, the shift from ANOVA repeated measure over time to ANOCOVA.

<ul style="list-style-type: none"> ▼ Purchase of male adult Sprague Dawley rats ▼ Acclimatization of animals: water, pigeon pellets given ad libitum 15 male rats, 8-month old, 300 g weight ▼ Overnight fasting of animals ▼ Randomization of animals to 3 treatment groups (positive control, test drug, negative control) [completely randomized design] 	
<ul style="list-style-type: none"> ▼ 3 treatment groups / blood collection for serum uric acid (SUA) determination [blood collected via rat's tail, Povidone-iodine used as antiseptic] 	
DAY 0	AFTER DAY 0
<ul style="list-style-type: none"> • Day 0 Hour 0 (= baseline) SUA • I. OXONATE-INDUCED HYPERURICEMIA Oxonate-urate challenge: <ul style="list-style-type: none"> - potassium oxonate 250 mg/kg body weight - uric acid 50 mg/kg body weight • After 3 hours Day 0 Hour 3 SUA • 1 mL distilled water given • water, food resumed 	<ul style="list-style-type: none"> • Oxonate-urate challengeDay 1 up to Day 14 • After 1 hour II. URATE-LOWERING THERAPY ..Day 1 up to Day 14 <ul style="list-style-type: none"> - Positive Control : allopurinol 60 mg / kg body weight - Test Drug : <i>P pellucida</i> 1 g / kg body weight - Negative control : NSS 0.9% • After 2 hours Hour 3 SUADays 1, 7, 14 • 1 mL distilled water given • water, food resumed

Figure 1. Urate - lowering activity flow chart