National Journal of Physiology, Pharmacy and Pharmacology

RESEARCH ARTICLE

Urate-lowering effect of *Manilkara zapota* aqueous leaf extracts in a murine model of hyperuricemia

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Received: October 05, 2017; **Accepted:** November 09, 2017

ABSTRACT

Background: Hyperuricemia and diseases related to hyperuricemia have been recognized among Filipinos in different countries. Diseases related to hyperuricemia such as gout and increased risks of cardiovascular diseases have given an avenue for methods on lowering uric acid levels. **Aims and Objectives:** *Manilkara zapota*, commonly known as Chico, has been reported to have the potential to reduce the uric acid level and thus was tested using aqueous leaf extracts of the plant. **Materials and Methods:** The plant was administered at a low dose of 1g/kg body weight (BW) and a high dose of 3g/kg BW on 10–16-week-old male ICR mice for 28 days. Three control groups were used for comparison of results: The sham group was administered with sterile distilled water and was not treated with $KBrO_3$, a positive control group was administered with ascorbic acid instead of the leaf extract and was treated with $KBrO_3$, and a negative control group was administered with sterile water and was also treated with $KBrO_3$. Hyperuricemia was induced using KBrO3 on the last day to detect for uric acid lowering activity. **Results:** Analysis shows that the negative control group had the highest mean of uric acid levels after hyperuricemia induction resulting to a significant difference from among the other groups. The mean uric acid levels of the groups treated with the leaf extract and ascorbic acid after hyperuricemia induction did not have any significant difference from each other. **Conclusion:** There is a urate-lowering activity from *Manilkara zapota* aqueous leaf extracts that are possibly due to the presence of flavonoids.

KEY WORDS: Anti-uricemic; Blood Uric Acid; *Manilkara zapota*; *Mus musculus*

INTRODUCTION

Hyperuricemia is characterized by an elevated serum urate (>6–7 mg/dL) and has been found to be an increasing problem among Filipinos and elsewhere in the world.[1,2] Nowadays, people are closer to an unhealthy and dangerous lifestyle where diseases can also be attributed. More recently, aside from the risk factors of sex (male over female) and relatively high consumption of red meat, other risk factors have also been attributed to gout such as metabolic syndromes, which includes diabetes, hypertension, dyslipidemia, obesity, renal diseases, and use of drugs such a diuretics, aspirin, and cyclosporine.[1] This is verified in a study by Li-Yu[3] that found that more men are reported to be hyperuricemic than women, with a 37.8% and 18% prevalence, respectively, among Filipinos. It was made clear also that food rich in purine contributed strongly to the hyperuricemic effect.[3] Consuming purine-rich foods such as beer, seafood, and red meat contributes to the risk of having high uric acid levels in the blood occasionally.[4] Thus, it is important to seek alternatives or preventive measures such as proper diet and regulation of purine-rich food.

Although many medications may be prescribed to a person already suffering the disease, prevention through accessible and natural alternatives such as medicinal plants could

Access this article online	
Website: www.njppp.com	Quick Response code
DOI: 10.5455/njppp.2017.7.1039309112017	

be explored.[5] Plants such as chico contain stimulants and anti-inflammatory properties that are mostly associated with urate-lowering effects, of which may be used to treat conditions related to uric acid such as gout after further analysis of its effects.[6] Purines are found in high concentrations in meat products and is one of the main contributors of increasing uric acid levels when metabolized in the body;[4] thus, highlighting the significance to know the possible medical benefits the leaf of Chico may possess considering the dosage used. Due to the facts on hyperuricemia cases and taking into consideration the evolving lifestyle of individuals, this study remains significant to incorporate new and feasible products from leaf sources that have a potential for enhancing bodily functions. Through these healthy options, people will find it easy and affordable to improve their health conditions.

The study is regarded to be significant due to rising demand of natural alternatives of medicines, especially leaves for tea. Chico contains phytochemicals that may contribute specifically to this part of the study about serum uric acid levels.[6-9] This study is mainly significant to unlock its potential as a source of medically important properties for certain diseases giving way to new and natural preventive options for diseases associated to high uric acid levels. Thus, it becomes relevant to know what the benefits of *Manilkara zapota* aqueous leaf extract has on uric acid levels as well as to know what causes the potential property of the plant to lower uric acid levels if there are any.

MATERIALS AND METHODS

Procurement of Animals

Twenty-five 10–16-week-old male ICR mice were bought from the Food and Drug Association in Muntinlupa city and were housed individually at room temperature (27 ± 2°C) having 58% humidity with controlled 12:12 h of light/dark cycle. The mice were acclimatized in laboratory conditions for at least 7 days. The study was conducted in the animal house in De La Salle University where the temperature is controlled by the air conditioner, proper ventilation with the use of an exhaust fan, and a dim and quiet environment was conserved. All experimental procedures were approved by the Institutional Animal Care and Use Committee of De La Salle University following the standard guidelines for animal care as recommended by the Philippine Association of Laboratory Animal Science and the Department of Agriculture Bureau of Animal Industry. The experiment from extraction to the administration of samples and extrapolation of results was performed from September 2014 to April 2015.

Preparation of Plant Extracts

The collected Chico leaves from Paranaque city, Philippines, were verified by Mr. Danilo N. Tandang of the National Museum, Philippines. It was then oven-dried for at least 8 h with a gradual increase in temperature from 50°C to 120°C. Its powdered form was achieved using an Osterizer. After which, the powdered leaves were submerged in distilled water for at least 1 day. The first filtration of the submerged leaves was done to collect only the aqueous solution from the leaves. A second filtration was performed to filter out impurities from the first filtrate. The second filtrate was evaporated in a boiling water bath until only <75 mL of the deep brown extract was left in the Erlenmeyer flask for further extraction through lyophilization. The whole process of leaf extraction took at least 1 month before the administration of the treatments.

Experimental Proper

The experiment was conducted for 28 days after the mice have been acclimatized to the environmental conditions at the animal house. The mice were fed daily with the treatments using oral gastric gavage at around 11 am for 4 weeks. Each mouse was randomly assigned to five different groups with each group having five mice. Groupings for the 25 mice and the dosage of the treatments given are shown in Table 1. The sham group received a daily dosage of 0.5 mL/100 g body weight (BW) of sterile water for 28 days without $KBrO_3$ treatment on the 28th day. This group served as the basis for the normal results from the rest of the groups that were given treatments and induced with hyperuricemia using $KBrO_3$.

The leaf extracts were administered to two different groups, 1 g/kg BW and 3 g/kg BW, positive control received ascorbic acid, while negative control and sham group were administered with sterile water. The negative control group was given the same dosage as the sham group but was administered with 200 mg/kg BW $KBrO_3$ on the last day of treatment. All groups treated with the Chico aqueous leaf extract, and positive and negative controls were administered with 200 mg/kg BW $KBrO_3$ on the last day of treatment.

Blood sample collections were done on the 1st day of experimentation, the 1st day of the 3rd week before administering the treatment for the day, and twice on the last day of the last week (one 75 min after treatment for the last day and the other, 3 h after $KBrO_3$ administration) by tail nick method, and uric acid levels were measured using Easy Touch GCU.

Table 1: Groupings, treatment, and dosage per group

Groups	Treatment	Dosage
Sham group	Sterile water	0.5 mL/100 g BW
Negative control	Sterile water	0.5 mL/100 g BW
Positive control	Ascorbic acid	3 g/kg BW
Low dose	Chico leaf extract	1 g/kg BW
High dose	Chico leaf extract	3 g/kg BW

BW: Body weight

Data Analysis

Uric acid measurements were analyzed using one-way ANOVA. The means were compared using Tukey's test to determine significant differences among the treatment groups at $P < 0.05$. All statistical analysis was performed using STATA v.12.

RESULTS

Results generated from the statistical analysis show that the there are no differences between the baseline, pre-treatment, and pre-$KBrO_3$ treatment. This shows that there is no hyperuricemia among the different groups before the induction of $KBrO_3$. It can then be seen that the negative control group's mean uric acid levels 3 h after $KBrO_3$ treatment are significantly different from the other groups' mean uric acid levels. Among the different groups, the negative control had the highest postinduction of $KBrO_3$ mean. The equal and lower means in uric acid levels of the treatment and positive control groups 3 h after $KBrO_3$ may imply that urate-lowering activity is exhibited the Chico leaf extract. This finding could be indicative of the effectiveness of the Chico aqueous leaf extract [Table 2 and Figure 1].

DISCUSSION

According to Choi *et al.*,[10] men with high consumption of ascorbic acid, or Vitamin C, have lowered blood uric acid levels. Other studies on animal models and *in vitro* tests have shown the ability of ascorbic acid to increase uric acid excretion and to inhibit xanthine oxidase and consequently lower serum uric acid levels. Other studies performed on man demonstrated that the relationship between plasma ascorbic acid levels and serum uric acid levels has an indirect proportionality.[11] Ascorbic acid, having a significantly lower mean of uric acid levels 3 h after $KBrO_3$ treatment from the negative control group, reflected the same property of lowering uric acid levels. Although there were no significant differences from the uric acid level means for the positive control, there was an observed increase in the mean uric acid level 3 h after $KBrO_3$ treatment. This group which had ascorbic acid as treatment appeared to have low or no effect in lowering uric acid levels after 3 h postinduction of $KBrO_3$ compared to the two groups treated with *Manilkara zapota* leaf aqueous extract. Since Vitamin C is water soluble, it may be the reason that it only stays for a short period of time in the blood plasma then is excreted in the urine, which is why it is taken in high amounts.[12] In comparison to flavonoids that also stays in the body for a short period of time, it attracts toxins however (e.g., free radicals formed from purines) as it is secreted in the urine.[13] This may be the reason why that 3 h after administration of $KBrO_3$, *Manilkara zapota* leaf aqueous extract-treated groups showed a significant decrease of uric acid compared with the positive group.

Manilkara zapota, a rainforest plant that has become of economic importance, is now being cultivated in tropical countries for fruit production.[14] Its versatility to grow in different climates such as dry subtropical areas and wet tropics gives its wide distribution from Tropical America where it originated from to Southeast Asia through the Philippines. Its ecological requirements such as moist hot climate, temperatures of 10–38°C, and its favored growth in coastal regions give the Philippines an advantage in cultivating a tree that offers not only its marketable fruit but also its strong potential for medicinal supplements. Phytochemical analysis of *Manilkara zapota* leaf extract has confirmed the presence of alkaloid, flavonoid, tannin, and saponin contents in leaf extracts.[7] These compounds found in the leaf extract are what give the strong potential of the plant for medicinal uses. The lowered results of the

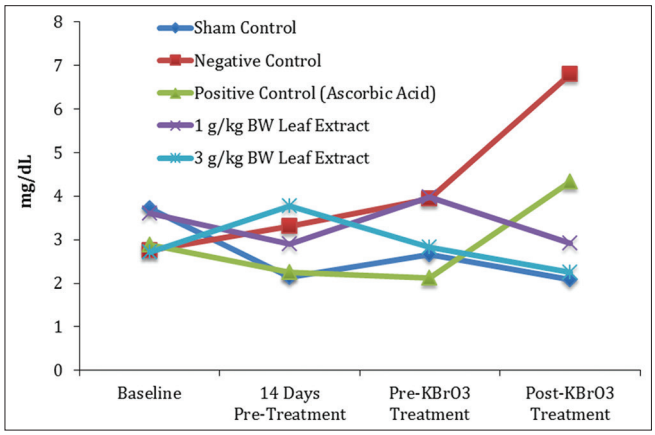

Figure 1: Graph showing the mean serum uric acid levels in the different treatments

Table 2: Mean and SD of uric acid levels in 28 days

Group	Baseline	14 days pre-treatment	28 days pre-treatment	
			Pre $KBrO_3$ treatment	3 h after $KBrO_3$ treatment
Sham control	3.72±1.61	2.14±0.19	2.66±1.31	2.08±0.13[1]
Negative control	2.76±0.82	3.30±1.05	3.94±1.05	6.80±1.12
Positive control	2.88±1.43	2.26±0.43	2.12±0.22	4.32±2.03[1]
1 g/kg BW	3.60±1.65	2.90±1.02	3.98±1.23	2.92±0.71[1]
3 g/kg BW	2.72±1.84	3.78±3.69	2.82±1.83	2.26±1.23[1]

Columns with the same superscript have no significant difference at $P<0.05$. BW: Body weight, SD: Standard deviation

group from the leaf extracts can be attributed to flavonoids. Flavonoids, like ascorbic acid, have been reported to lower uric acid levels due to its inhibitory property toward xanthine oxidase. From the study of Omar *et al.,*[15] flavonoids bind to three binding sites on the xanthine oxidase, and two of these binding sites are essential in the flavonoid inhibition of xanthine oxidase. Near the molybdopterin cofactor, one of the binding sites to which flavonoids are bound to is responsible for the xanthine oxidation, resulting to the uric acid product. To study the xanthine oxidase inhibition by flavonoids, Lin *et al.*[16] compared the binding of apigenin (a flavone) to the active site of xanthine oxidase (molybdopterin cofactor) with allopurinol which is a known inhibitor of the enzyme. By doing so, it was seen that the flavone exhibited the same docking to the molybdopterin cofactor as allopurinol did. The other binding site is the hydrophobic residues of the enzyme. The phenolic group of the flavone extended to the areas of xanthine oxidase with hydrophobic residues. This hydrophobic interaction stabilizes the flavone to the molybdopterin cofactor. The same positioning of quercetin (a flavonol) was observed only with a weaker interaction. It is the active site binding activity and the hydrophobic interactions with xanthine oxidase that affect the potency of the inhibitory capacity of flavonoids.[15] As demonstrated by Lin *et al.,*[16] some subgroups of flavonoids have been shown to have high inhibitory activity on xanthine oxidase such as chalcone flavonoids, planar flavones, and flavonols bearing a 7-hydroxyl group. Flavonoids in a chalcone form have higher inhibitory effects on xanthine oxidase than flavonoids in the closed ring form.[17] Lee *et al.*[18] have also observed results of chalcones strongly inhibiting xanthine oxidase activity. The results of Nagao *et al.*[19] have evidence that inhibition of xanthine oxidase activity is achieved at low concentrations of planar flavones and flavonols. These studies support the results that the urate-lowering activity of the leaf extracts of *Manilkara zapota* is due to the presence of flavonoids. The extent of effectiveness of the leaf extract over ascorbic acid shows no difference, which may be due to the boiling process of attaining the leaf extract since degradation of phenolic compounds increases as temperature increases,[20] and thus, the flavonoid activity in the extract could have been reduced. Finally, results show that there is no significant difference between the mean uric acid levels of the groups treated with 1 g/kg BW and 3 g/kg BW 3 h after $KBrO_3$ treatment. This may indicate that the urate-lowering effect of *Manilkara zapota* aqueous leaf extract is not dose dependent.

CONCLUSION

The administration of aqueous leaf extract of *Manilkara zapota* for urate lowering properties on 25 10–16-week-old male albino mice for 28 days showed that the uric acid levels among the groups treated with the leaf extract and ascorbic acid have demonstrated lowered uric acid levels after hyperuricemia induction by $KBrO_3$. Furthermore, the negative control group had statistically higher uric acid levels after hyperuricemia induction when compared with the treatment groups and positive control. Such results suggest that the leaf extract of *Manilkara zapota*, like ascorbic acid, has urate-lowering properties, which may be attributed to the plant's flavonoid content. Other than the not statistically significant differences between the positive control and the treatment controls 3 h after $KBrO_3$ administration, the uric acid level means of the 1 g/kg BW and 3 g/kg BW 3 h after $KBrO_3$ treatment are also not significantly different. Thus, it may be deduced that the aqueous leaf extract of *Manilkara zapota* has urate-lowering properties that are not dose-dependent. However, since no significant difference was seen between the means of the leaf extracts and ascorbic acid, there is no evidence to construe that the aqueous leaf extract of *Manilkara zapota* has a greater uric acid-lowering ability than ascorbic acid and vice versa. Thus, in lowering uric acid levels, ascorbic acid and leaf extract have the same extent of its urate lowering activity.

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How to cite this article: Cervero MGG, Sy JED, Ples MB, Vitor RJS II. Urate-lowering effect of *Manilkara zapota* aqueous leaf extracts in a murine model of hyperurecemia. Natl J Physiol Pharm Pharmacol 2018;8(3):426-430.

Source of Support: Nil, **Conflict of Interest:** None declared.