IN-VIVO EVALUATION OF THE PREVENTIVE EFFECT OF THE ETHANOLIC LEAF EXTRACT OF *HIBISCUS ROSA-SINENSIS* LINN. (MALVACEAE) AGAINST CALCIUM OXALATE CRYSTALS IN ETHYLENE GLYCOL AND AMMONIUM CHLORIDE-INDUCED HYPEROXALURIA IN ADULT MALE ALBINO SWISS MICE


University of Santo Tomas Faculty of Pharmacy España Blvd, Manila, Philippines, 1008.

ABSTRACT

Urolithiasis, the formation of stones that is caused by the supersaturation of urine. *Hibiscus rosa-sinensis* Linn. (Malvaceae), known as Gumamela in the Philippines, is known for having numerous pharmacologic uses. Having the same genus as *Hibiscus sabdariffa*, a proven anti-urolithiatic agent, the researchers choose Gumamela as the plant to be evaluated for its anti-urolithiatic activity. Thirty-six male Albino Swiss mice were randomly divided into 6 equal groups. Group I served as the normal group. Groups II, III, IV, V, and VI received ethylene glycol (0.75% v/v) and ammonium chloride (2%) from day 4 up to day 10. Group II served as the negative control. Alongside, group III (positive control) received 25mg/kg Hydrochlorothiazide; groups IV, V, and VI (preventive groups) received 250mg/kg, 500mg/kg, and 1000mg/kg of ethanolic leaf extract respectively from day 1 up to day 10, considering the first three days as prophylaxis. The mice’s 24-hour urine samples were collected on day 0, 3 and 10 have been examined for the presence of calcium oxalate (CaC₂O₄) crystals. On the 10th day, sub-mandibular blood collection was used to collect for testing of biochemical parameters. The urine crystals of the group I (p=0.105), group III (p=0.368), group IV (p=0.097) and group V (p=0.174) did not have significant changes. However, the urine crystals of the group II significantly increased
(p=0.022), specifically at day 10 while the urine crystals of the group VI had continuous decrease (p=0.016) from baseline to day 10. The decrease in calcium oxalate crystallization was due to the ability of the ethanolic leaf extract to prevent urinary supersaturation of calcium oxalate in a dose dependent manner. To conclude, *Hibiscus rosa-sinensis* Linn. (Malvaceae) ethanolic extract has a significant therapeutic potential for the prevention and inhibition of the formation of CaC$_2$O$_4$ induced crystals.

**KEYWORDS:** Urolithiasis, Calcium Oxalate, Blood Urea Nitrogen, Serum Creatinine, Swiss Albino Mice.

**INTRODUCTION**

Urolithiasis, the formation of low-density crystals is caused by the supersaturation of the urine. These crystals consist of calcium oxalate, magnesium ammonium phosphate, cystine, and uric acid. Significant growth of these crystals will cause excruciating pain and inhibit the flow of urine due to the blockage of the ureter. No oral treatment for urolithiasis is currently available, however, invasive procedures are available but costly. Thiazide diuretics are normally used as an adjunct therapy for urinary stones as an aid in reducing the urinary calcium concentration. The significance of this study is to provide an alternative and cheaper treatment for urolithiasis and for the improvement of the quality of life of individuals suffering from the said condition.

*Hibiscus rosa-sinensis* Linn. (Malvaceae), commonly known as Gumamela in the Philippines, is traditionally used as an anti-oxidant, anti-amnesic, antityrosinase, antidyskenesia, hypolipidemic, hepatoprotective, hair growth stimulating, wound healing, anticonvulsive, antimicrobial, antipyretic, and antimutagenic (Lim, 2014). Having the same genus as *Hibiscus sabdariffa*, a proven anti-urolithiatic agent, the researchers agreed on choosing Gumamela as the plant sample to be investigated for its anti-urolithiatic activity. So far there are limited scientific studies on anti-urolithiatic effects of *Hibiscus rosa-sinensis* under *in-vivo* conditions.

The study assessed the preventive effect of *Hibiscus rosa-sinensis* Linn. (Malvaceae) against calcium oxalate crystal formation on ethylene glycol and ammonium chloride-induced adult, male, Swiss albino mice.
MATERIALS AND METHODS

Collection of *Hibiscus rosa-sinensis* (Malvaceae) Leaves

The leaves of *Hibiscus rosa-sinensis* (Malvaceae) were collected from Lupao, Nueva Ecija, Philippines on April 5, 2015. The pharmacognostic authentication of the *Hibiscus rosa-sinensis* (Malvaceae) leaves was done by experts from the University of Santo Tomas Research Center for Natural and Applied Science (RCNAS) Herbarium in Thomas Aquinas Research Complex in University of Santo Tomas, Sampaloc, Manila, Philippines.

Preparation of the plant extract

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Hibiscus rosa-sinensis</em> leaves were washed and air-dried.</td>
</tr>
<tr>
<td>2</td>
<td>The air-dried leaves were pulverized using the Wiley mill.</td>
</tr>
<tr>
<td>3</td>
<td>The weight of the pulverized leaves was obtained.</td>
</tr>
<tr>
<td>4</td>
<td>The leaves were macerated with enough amount of Ethanol and percolated.</td>
</tr>
<tr>
<td>5</td>
<td>The extract was allowed to stand for 24 hours and was collected every other day.</td>
</tr>
<tr>
<td>6</td>
<td>The ethanolic extract was subjected to rotary evaporation until all the solvent evaporated.</td>
</tr>
</tbody>
</table>

**Figure 1: Preparation of the Crude Extract**

The collected leaves were carefully washed with water to remove unnecessary dirt and were subjected to air-drying for 4 weeks. The dried sample was finely pulverized through the use of a Wiley mill and the powdered dried leaves were weighed. The sample was subjected to extraction using a percolator apparatus to obtain the organic constituents from the leaves. In the percolation set-up, a sufficient amount of 95% Ethanol was used to moisten and saturate the sample. The tap at the bottom part of the apparatus was kept open for the occluded gas to pass out. Furthermore, 1000 milliliters of 95% Ethanol was poured over the sample and allowed to stand for 24 hours. The set-up was allowed to work and the filtrate was collected every other day. The process continued until 2 extractions were made. After extracting, the ethanolic extract was placed in a rotary evaporator to facilitate the evaporation of the solvent and to retrieve the crude extract.

**Chemicals and apparatus:** Ethylene glycol (0.75% v/v) and ammonium chloride (2%) was purchased at Laboratory Equipment and Supplies Office (LESO) of University of Santo Tomas. All other chemicals and reagents used were laboratory grade and procured from approved chemical suppliers. Apparatus such as Wiley mill, rotary evaporator, metabolic cage, compound microscope, and centrifuge were used in the study.
Animals: Adult, male, Swiss albino mice (body weight of 25-40g) were used for the present study. They were housed in steel cages and maintained under controlled lighting and temperature. The animals were given standard diet. The study protocol was approved by the Research Center for Natural and Applied Sciences in accordance with the rules and guidelines of the Institutional Animal Care and Use Committee.

Qualitative Phytochemical Analysis: The extract was tested for the presence of bioactive compounds by using following standard methods: Test for Flavonoids.

Lead Acetate Test: Sample was mixed with lead acetate solution. Yellow precipitate formation indicated the presence of flavonoids.

Sample Preparation: Ten grams of the crude extract was defatted by adding 9 mL of hexane and 4.5 mL of water. The upper hexane layer was discarded. The retained defatted aqueous layer was mixed with 10 mL of 80% ethanol. The solution was filtered and divided into three portions. One portion served as control and the remaining two were subjected to Bate-Smith and Metcalf’s test and Wilstater “Cyanidin” test.

Bate-Smith and Metcalf’s Test: One portion of the solution was added 0.5 mL of 12M of concentrated hydrochloric acid. The solution was subjected to water bath for 15 minutes. Red or violet coloration of the solution within an hour confirmed the presence of Leucoanthocyanin therefore positive for Bate-Smith and Metcalf’s test.

Wilstater “Cyanidin” Test: Another portion of the solution was added 0.5 mL of concentrated hydrochloric acid and 4 pieces of magnesium turnings. Then, an equal volume of water and 1 mL of Octyl alcohol were mixed to the solution and was left to stand for a few minutes. Green to blue coloration indicated positive for Wilstater test.

Pharmacologic Evaluation
Renal calculi induction and treatment: Urolithiasis was induced by ethylene glycol (0.75% v/v) and ammonium chloride (0.2%) in experimental animals. Thirty-six adult male Swiss Albino mice were randomly divided into 6 groups (n=6). All the animal groups received food and distilled water. The groupings are as follows: Group 1, the normal control, was given an ad libitum access to regular food and distilled water. Group 2 was given 1ml of 0.75% [v/v] ethylene glycol and 2% ammonium chloride to induce hyperoxaluria for 7 days using oral gavage. Group 3 was given 0.15ml of 10mg/kg/ml of hydrochlorothiazide daily as
prophylaxis for 3 days before the induction of hyperoxaluria. Thereafter, 0.15ml containing 0.75% ethylene glycol and 2% ammonium chloride was given daily along with the treatment of 0.15ml of 10mg/kg/ml of hydrochlorothiazide daily from day 4 to day 10 using oral gavage. Groups 4, 5, and 6 were given of low (250mg/kg), intermediate (500mg/kg), and high (1000mg/kg) dose of crude extract daily respectively as prophylaxis for 3 days before the induction of hyperoxaluria. Thereafter, 0.15ml containing 0.75% ethylene glycol and 2% ammonium chloride were given daily along with the treatment of low (250mg/kg), intermediate (500mg/kg), and high (1000mg/kg) dose of crude extract daily to groups 4, 5, and 6 respectively from day 4 to day 10 using oral gavage.

**Diagnosis of specific urine particles:** The animals were paced in individual metabolic cages and 24-hour urine samples were collected on day 0, 3, 10 of calculi induction preventive treatment period. The urine samples were examined for the presence of calcium oxalate crystals.

**Renal function test:** On the 10th day, blood was collected for biochemical parameters, specifically to determine if there is an increase or decrease of serum creatinine and blood urea nitrogen (BUN).

**Kidney histopathological study:** All the test animals were sacrificed and their kidneys were harvested after euthanasia. Kidney tissues were longitudinally trimmed for better visualization of the kidney structure. The histological stains used were Hematoxylin, a nuclear stain and Eosin, a cytoplasmic counterstain. The tissues were then viewed under compound microscope and histopathologically evaluated for presence of calcium oxalate crystals.

**Ethical Considerations:** Before the experiments were conducted to the test animals, the researchers made sure that the protocols have been approved by the University of Santo Tomas Institutional Animal Care and Use Committee. The researchers also made sure that the conducted protocols were done in accordance to The Republic Act No. 8485, more commonly known as the Animal Welfare Act of 1998, which protects and upholds the welfare of the animal subjects by supervising and regulating the establishments and operations of all facilities and equipment that were used in biomedical testing. Before proceeding with the protocols, each researcher was well-trained and well-equipped with the knowledge about the procedures. They worked in accordance to the ethical considerations.
Everything that was tested and done to the Swiss Albino Mice were in accordance to the approved protocol and has been studied well to avoid any complications and suffering of the test subjects.

Analysis

Data interpretation and Calculations: Repeated Measures Analysis of Variance with Post hoc of Tukey’s Honest Significance Test was used to determine the significant difference between the urine volumes of each group. For the analysis of the urinary crystals, Friedman Test was used for each group’s trend; Kruskal-wallis ANOVA for the comparison of groups in each day. For the blood parameters (BUN and Serum Creatinine), Single Factor Analysis of Variance with the post hoc of Tukey’s Honest Significance Test was used.

RESULTS

Qualitative Phytochemical Analysis: The test for flavonoids all yielded positive results. Lead Acetate Test yielded yellow precipitate. Bate-Smith and Metcalf’s Test formed violet coloration of the sample solution. Wilstater “Cyanidin” Test produced blue green coloration of the solution.

Urine Volume: For the urine volume, the statistical test used was Repeated Measures Analysis of Variance and Tukey’s HSD as the post hoc analysis. The mean volume of the urine of the mice on the baseline, day 3 and day 10 are shown in Table 1 and Figure 2.

![Figure 2. Mean of Urine Volume (Baseline, Day 3, Day 10 of collection)](image-url)
Table 1. Mean of Urine Volume (Baseline, Day 3, and Day 10 of collection)

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Day 3</th>
<th>Day 10</th>
<th>F stat</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>0.75 ± 0.03</td>
<td>0.73 ± 0.02</td>
<td>0.77 ± 0.03</td>
<td>0.614</td>
<td>0.560</td>
</tr>
<tr>
<td>Group 2</td>
<td>0.77 ± 0.05</td>
<td>0.77 ± 0.03</td>
<td>0.44 ± 0.02</td>
<td>104.576</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group 3</td>
<td>0.74 ± 0.04</td>
<td>1.69 ± 0.09</td>
<td>1.05 ± 0.07</td>
<td>54.357</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group 4</td>
<td>0.79 ± 0.04</td>
<td>0.87 ± 0.03</td>
<td>0.61 ± 0.01</td>
<td>31.393</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group 5</td>
<td>0.74 ± 0.02</td>
<td>1.67 ± 0.06</td>
<td>1.74 ± 0.03</td>
<td>276.347</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Group 6</td>
<td>0.64 ± 0.04</td>
<td>1.77 ± 0.12</td>
<td>1.94 ± 0.1</td>
<td>150.040</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM, n = 6.

Group 1 (p = 0.560) did not have significant changes in their mean urine volume on the baseline, Day 3 and Day 10 of treatment. Group 2 did not have changes on the baseline and Day 3 (p = 1.000), but their urine volume decreased at Day 10 (p<0.001). Likewise, Group 4 did not have changes on the baseline and Day 3 (p=0.135), but their urine volume decreased significantly at Day 10 (p=0.002). On the other hand, Group 3 had a significant increase in their urine volume on the baseline and Day 3 (p<0.001) but significantly decreased their urine volume at Day 10 (p=0.005). Groups 5 and 6 had a significant increase on the baseline and Day 3 (p<0.001) but did not have significant changes at on Day 10 (p>0.05).

At baseline, the mean urine volume of the six groups did not differ [F = 2.044, p=0.101]. But at day 3, the mean urine volume of the six groups significantly differ [F=54.012, p<0.001] in which Groups 3, 5 and 6 (p=0.927) did not differ. The mean urine volume of Groups 1, 2 and 4 did not differ as well (p=0.745). But groups 3, 5, and 6 had significantly higher (p<0.05) mean urine volume as compared to Groups 1, 2 and 4.

At day 10, the mean urine volume of the six groups significantly differ [F=122.913, p<0.001] in which Group 6 did not differ with the Group 5 (p=0.161), having significantly the highest mean urine volume. Group 3 (p<0.001) had significantly less urine volume versus Groups 5 and Group 6, but significantly higher urine volume as compared with Group 1 (p=0.015). Groups 2 and 4 did not differ (p=0.334) too but they have significantly less (p<0.05) mean urine volume.

Overall, the mean urine volume of the six groups significantly differ [F=62.718, p<0.001] in which Groups 1, 2 and 4 did not differ (p = 0.692). But the mean urine volume of Group 3 is significantly higher than Groups 1, 2, and 4 (p<0.001). The mean urine volume of Groups 5 and 6 did not differ (p=0.925), but is significantly higher than Group 3 (p<0.05).

Renal Calculi Excretion Results: The statistical tests used for the comparison of the calcium oxalate stones are Friedman Test for each group’s trend and Kruskal-wallis ANOVA.
for comparison of groups on each day. The calcium oxalate stones median scores on the baseline, Day 3, and Day 10 are shown in Table 2.

**Table 2. Calcium Oxalate Stones Median Scores (Baseline, Day 3, Day 10)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Day 3</th>
<th>Day 10</th>
<th>$\chi^2$ stat</th>
<th>$p$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>1.5 [1.0 to 2.0]</td>
<td>1.5 [1.0 to 2.0]</td>
<td>1.0 [1.0 to 1.0]</td>
<td>4.500</td>
<td>0.105</td>
</tr>
<tr>
<td>Group 2</td>
<td>1.0 [1.0 to 2.0]</td>
<td>1.0 [1.0 to 1.0]</td>
<td>2.0 [1.8 to 2.0]</td>
<td>7.600</td>
<td>0.022</td>
</tr>
<tr>
<td>Group 3</td>
<td>2.0 [1.8 to 3.0]</td>
<td>2.0 [1.8 to 2.0]</td>
<td>2.0 [1.8 to 2.0]</td>
<td>2.000</td>
<td>0.368</td>
</tr>
<tr>
<td>Group 4</td>
<td>2.0 [2.0 to 3.0]</td>
<td>2.0 [1.8 to 2.3]</td>
<td>2.0 [1.8 to 2.0]</td>
<td>4.667</td>
<td>0.097</td>
</tr>
<tr>
<td>Group 5</td>
<td>2.5 [1.8 to 3.0]</td>
<td>2.0 [1.0 to 3.0]</td>
<td>2.0 [1.0 to 2.0]</td>
<td>3.500</td>
<td>0.174</td>
</tr>
<tr>
<td>Group 6</td>
<td>2.5 [2.0 to 3.0]</td>
<td>1.5 [0.8 to 2.0]</td>
<td>1.0 [1.0 to 1.3]</td>
<td>8.316</td>
<td>0.016</td>
</tr>
</tbody>
</table>

Values expressed as median [IQR].

The codes used were: 3: +++; 2: ++; 1: +; and 0: -

The codes used for the statistical analysis of the urine crystals are 3 for +++ , 2 for ++ , 1 for +, and 0 for – crystals. The urine crystals of Group 1 ($p=0.105$), Group 3 ($p = 0.368$), Group 4 ($p =0.097$) and Group 5 ($p =0.174$) did not have significant changes. But the urine crystals of Group 2 significantly increased ($p=0.022$), specifically at Day 10, while the urine crystal of Group 6 had continuous decrease ($p=0.016$) from baseline up to Day 10.

However, it was found that at baseline [$\chi^2 = 13.107, p = 0.022$], the urine crystals already differ, in which Group 2 already had significantly the least urine crystals. But the remaining five groups did not differ ($p=0.069$). At Day 3, the urine crystals do not differ [$\chi^2 = 10.893, p = 0.054$]. But at Day 10, the urine crystals significantly differ [$\chi^2 = 16.625, p = 0.005$], in which Group 1 and Group 6 did not differ ($p=0.470$), but had significantly less urine crystals as compared to the other four groups. The urine crystals of the groups 2, 3, 4, and 5 did not differ ($p=0.883$) at Day 10.

**Blood Chemistry**

**Blood Urea Nitrogen:** In determining the mean of the Blood Urea Nitrogen (BUN) of the mouse groups, the statistical test used was Single Factor Analysis of Variance, and Tukey’s HSD as the post hoc analysis. Shown in Table 3 and Figure 3 are the mean of the mouse groups’ BUN.
Normal reference range for BUN: 8 to 33 mg/dL

**Figure 3. Mean of Blood Urea Nitrogen**

**Table 3. Mean of Blood Urea Nitrogen**

<table>
<thead>
<tr>
<th>Group</th>
<th>BUN</th>
<th>F stat</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31.9 ± 1.4</td>
<td>1.810</td>
<td>0.141</td>
</tr>
<tr>
<td>2</td>
<td>36.1 ± 3.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>34.3 ± 4.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>28.1 ± 2.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>25.7 ± 3.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>28.4 ± 0.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values expressed as mean ± SEM, n = 6.

There is no significant difference in the mean BUN \( F = 1.810, p = 0.141 \) of the six groups. The mean BUN of Groups 1, 4, 5 and 6 are in the normal range. Although the mean BUN of Group 2 \( p=0.394 \) and Group 3 \( p=0.793 \) are beyond the normal reference range, their mean BUN did not significantly differ with 33 mg/dL; this means that their BUN level can still be considered normal since their \( p \)-values are greater than 0.05.

**Serum Creatinine**: In determining the mean of the serum creatinine of the mouse groups, the statistical test used was Single Factor Analysis of Variance, and Tukey’s HSD as the post hoc analysis. Shown in Table 4 and Figure 4 are the mean of the mouse groups’ serum creatinine.

**Table 4. Mean of Serum Creatinine**

<table>
<thead>
<tr>
<th>Group</th>
<th>Creatine</th>
<th>F stat</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.47 ± 0.04</td>
<td>0.963</td>
<td>0.456</td>
</tr>
<tr>
<td>2</td>
<td>0.51 ± 0.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.44 ± 0.04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.38 ± 0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.48 ± 0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.56 ± 0.06</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Values expressed as mean ± SEM, n = 6.

Normal reference range for Creatinine: 0.2 to 0.9 mg/dL.

There is no significant difference in the mean serum creatinine \[F = 0.963, p = 0.456\] of the six groups. Moreover, the mean serum creatinine of all the six groups are within the normal reference range.

**Kidney histopathological study:** There were no observable toxicity found on the kidney tissues of the following groups of test subjects.
DISCUSSION
Positive results for the qualitative tests indicates that *Hibiscus rosa-sinensis* contains flavonoid. A class of compound which is known to prevent oxidative stress which inhibits oxidation of ethylene glycol to oxalate.

The urine crystals of Groups 1, 3, 4, and 5 did not have significant changes. The urine crystals of Group 2 significantly increased, specifically at day 10, while the urine crystals of Group 6 had continuous decrease from baseline to day 10. To conclude, hydrochlorothiazide, 250mg/kg and 500mg/kg of ethanolic leaf extract of *Hibiscus rosa-sinensis* maintained the normal calcium oxalate level in comparison with group 1, which means that it can normalize the calcium oxalate crystal level. Furthermore, the urine crystals of Group 2 (negative control) significantly increased while the urine crystals of the ethanolic leaf extract with a dose of 1000mg/kg significantly decrease in comparison with Groups 1 and 2, which indicates that it reduces the risk of the formation of calcium oxalate crystals.

The mean blood urea nitrogen (BUN) of the control group and the groups treated with 250mg/kg, 500mg/kg & 1000mg/kg are within the normal range, whereas the mean BUN of the group that was induced with ethylene glycol and ammonium chloride, and the group treated with hydrochlorothiazide 25mg/kg are not within the preferred normal range. However, their mean BUN did not differ significantly from the four other groups. The mean creatinine of all the six groups in the study are within the normal reference range. Therefore, it is indicative that the *Hibiscus rosa-sinensis* (Malvaceae) plant extract did not affect the serum and BUN normal range whereas the hydrochlorothiazide and ethylene glycol and ammonium chloride may have been a factor in providing a slightly different result for the BUN.

CONCLUSIONS
In conclusion, the experimentation demonstrated that the ethanolic leaf extract of *Hibiscus rosa-sinensis* (Malvaceae) exhibited preventive effect against the formation of calcium oxalate crystals on the ethylene glycol- & ammonium chloride-induced adult male Swiss Albino mice in high doses. There was no significant change in the BUN and creatinine levels of the control group and of the *Hibiscus rosa-sinensis* plant extract treated groups; this suggests that there is neither complication nor kidney damage due to the plant extract.
ACKNOWLEDGEMENT

The success of this study required the help of various individuals. Without them, we might not meet our objectives in doing this study. We want to give our utmost gratitude to the people who invaluably helped and supported us all throughout our study. First, our praises and deepest gratitude to the Almighty God, who is the source of all – our knowledge, wisdom, strength, patience, talents and skills needed in the study.

Second, our supportive family, who motivates, inspires and encourages us to always do our best to complete the study, and who are always there for us comes the time that we need them.

Third, our thesis adviser and co-thesis adviser, Assist. Prof. May Tagoc-Magtoto, M.S. Phar and Prof. Aleth Therese L. Dacanay, Ph.D. respectively, for the patience and time spent with us, for all the knowledge, constructive criticisms, and open suggestions shared that greatly helped us in the study, and for supporting, motivating, and never giving up on us.

Fourth, our professors, especially Assoc. Prof. Rosario R. Aranda, for the ideas and pieces of advice shared which helped us improve the study.

Fifth, Instructor Christine Joy H. Acosta, M.S. Phar., for giving us views and suggestions about our research.

Sixth, Assist. Prof. Gregorio L. Martin, for helping us view the calcium oxalate crystals from the urine of Swiss albino mice Seventh, Assist. Prof. Xandro Alexi A. Nieto M.A. Educ. in Math, for helping us interpret, analyze the data and help us determine the right statistical design for each of the results gathered.

Lastly, to our group who poured a lot of effort, time, and hard work to finish the study, and to the people who served as our inspiration, especially our friends who helped and contributed ideas in the study. Our sincerest gratitude for without them, this study would not be possible.

REFERENCES

3. Animal Welfare Act 1998 (IRR) s.6 (PH.)