Preliminary investigation of the safe dose of oleandrin when administered orally to Beagles

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OBJECTIVE
The purpose of the study reported herein was to determine the dose of oleander extract and oleandrin (the key pharmacologically active constituent) that could be safely administered PO to dogs.

ANIMALS
42 purebred Beagle dogs were used to study an extract of Nerium oleander.

METHODS
3 studies were performed in 42 purebred young adult (ages 12 months or older) Beagle dogs using a supercritical fluid extract of Nerium oleander leaves. The first study was an 8-day initial dose-ranging study in 2 dogs, a second 7-day repeat-dosing study was performed in 4 dogs, and the final study was performed in 32 dogs where test subjects were given extract or placebo once daily for 28 consecutive days via oral (gavage) administration followed by a 14-day recovery period.

RESULTS
At 2.3 µg/kg of oleandrin, there were no observable adverse effects during the duration of the study. Adverse effects were not seen until doses exceeded 6.9 µg/kg of oleandrin, at which time mild, reversible clinical signs were noted. However, a dose > 460 µg of oleandrin/kg was fatal in 1 of 2 dogs in this study.

CLINICAL RELEVANCE
The studies reported here, taken in totality, suggest that doses exceeding 6.9 µg/kg of oleandrin may be associated with cardiac abnormalities. An estimated no treatment effective adverse event oral dose of oleandrin appears to be 4.6 µg of oleandrin/kg. Higher doses may be tolerable but should be used with appropriate monitoring.

Keywords: oleandrin, safety, oleander, cardiac, canine

Nerium oleander is a well-known botanical species that has been employed for centuries in traditional medicine due to the well-recognized therapeutic properties of constituent molecules known as cardiac glycosides. This flowering shrub, native to the Mediterranean region and parts of Asia, has a rich historical background in the context of its medicinal use.1–3 The present manuscript describes preliminary studies investigating the safe use and adverse effects of an oleander extract administered PO in dogs.

Cardiac glycosides are a class of natural compounds found in various plants, with digitals (from Digitalis purpurea, which is commonly referred to as foxglove) and oleandrin from Nerium oleander being notable examples. These compounds have been used in medicine for their ability to beneficially affect cardiac function, primarily by influencing the movement of ions across cell membranes in cardiac muscle cells, providing a more efficient pumping of heart muscle.4 Over the past 15 years, however, additional beneficial properties of oleandrin and defined extracts of Nerium oleander have been reported. These include the well-documented anticancer properties of oleandrin against malignancies in humans.5–10 More recently, oleandrin has been reported to be effective as both a prophylactic as well as therapeutic agent with broad antiviral effects against important enveloped viruses affecting humans11–15 as well as some viruses affecting cattle.16 In addition, oleandrin has been reported to facilitate and support a healthy immune function17 while also serving as an important anti-inflammatory agent.18 Recently, oleander was reported to be a potent senolytic.19
Like many, if not most, approved medicinal agents, a proper dose is required to achieve beneficial responses. Likewise, too high a dose may result in toxic consequences. This is especially true for cardiac glycosides that have been reported to have a narrow therapeutic window.\(^{26}\) This is evident when one considers the careful monitoring of digitalis blood levels required for safe use of this agent for treatment of congestive heart failure. As with digitals, high doses of oleandrin have also been associated with well-known toxic effects; however, clinical phase I and II studies have clearly demonstrated that extracts containing oleandrin can be safely administered to patients with malignant disease.\(^{21,22}\) Thus, an important understanding of the pharmacologic benefits versus risk of the use of oleandrin has now become evident.

Very few studies have been conducted to carefully explore the impact of oleandrin or oleander plant material or extract on animals. Reports of livestock poisonings are more often related to toxicity associated with feeding food that is contaminated with excessive amounts of oleander leaves or with accidental ingestion of excessive amounts of the plant from foraging raw material. Oleander leaves are bitter and typically are not eaten by livestock. Szabuniewicz et al\(^{23}\) examined oleander toxicity in monkeys, goats, mice, rats, and chickens. Similar to humans, dogs, cats, goats, and monkeys were found to be sensitive to the cardioactive glycosides extracted from dried oleander leaves. In contrast, rodent and avian species were observed to be insensitive to oleander cardioactive glycosides.\(^{23–25}\) This is important because safety studies in rodents appear to have little or no relevance to dogs.

Numerous documented cases have demonstrated the potential lethality of oleander ingestion in dogs when consumed in excessive amounts. Symptoms may include vomiting, diarrhea, drooling, and cardiac abnormalities. Interestingly, in 1 study\(^{26}\) of toxicity in a dog that consumed oleander leaves, a main presenting sign was hypoglycemia. While the toxicity of high doses of \(N\) \(oleander\) is known, medicinal uses of low doses have been less commonly discussed. The purpose of this manuscript is to describe new information concerning the safety of \(N\) \(oleander\) extracts in dogs and to report on doses of oleandrin that can be safely administered PO to dogs. Studies reported here used a supercritical fluid extract of oleander (PBI-05204). Data are reported regarding dose of extract and the amount of the active, oleandrin, in each dose. Importantly, the dog appears to be an excellent animal model to look at both the safety and therapeutic value of oleandrin for humans.

### Methods

For these studies, 38 purebred Beagle dogs aged 12 months or older and weighing between 7.6 and 13.4 kg (females, 7.6 to 8.9 kg; males, 10.6 to 13.4 kg) were used. These studies were performed by Charles River Laboratories, Preclinical Services, Arkansas. The studies complied with all applicable sections of the Final Rules of the Animal Welfare Act regulators (9 CFR). The protocols and amendments were reviewed and approved by the testing facility IACUC.

A PO route of administration was selected since this is the intended route of canine administration. Dogs were group housed (2/run) in separate chain-link runs with epoxy-coated concrete floors. Purina Certified Canine Diet No. PMI 5007 (Nestlé Purina) and filtered tap water (using a reverse osmosis system; Thermo Fisher Scientific) were provided ad libitum. The feed, water, and bedding were routinely analyzed for microbial and fungal contaminants. Animals were acclimated to gavage dosing with 8 mL of filtered tap water for 4 consecutive days prior to dosing and were examined by the staff veterinarian prior to being released for use in the study. Randomization was not required for these studies, and only naïve animals were used.

The supercritical fluid extract, PBI-05204, of \(N\) \(oleander\) leaves made available from Phoenix Biotechnology Inc (San Antonio, TX), was used in this study. This compound is neither FDA approved nor commercially available in the US. It has been well characterized and used safely in phase I and II human clinical trials.\(^{21,22}\) The content of oleandrin in the extract was determined by HPLC (phenyl-hexyl column run under isocratic conditions using Solvent A [0.1% trifluoroacetic acid] and Solvent B [0.1% trifluoroacetic acid in acetonitrile]; ratio of A:B is 70:30%) with UV detection (216 nm) and internal (oleandrin) and external (cinobufagin) calibration standards.

#### Study I: a dose range–finding study of oleander extract administered PO to Beagle dogs

To determine an initial safe starting dose for dogs, a dose range–finding (single-dose) study was conducted in Beagle dogs. One male and 1 female dog were administered escalating doses of 5, 10, and 20 mg/kg of PBI-05204 (equivalent to 115, 230, and 460 \(\mu\)g of oleandrin/kg, respectively), followed by a 3-day washout/observation period between doses. Dosing was with 5 mg/kg of extract on study day 1, 10 mg/kg of extract on study day 4, and 20 mg/kg of extract on study day 8 (Table 1).

### Table 1—Dosing of oleander extract in 2 Beagle dogs in a dose-ranging study.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of animals</th>
<th>Dose level (mg/kg)</th>
<th>Dose volume (mL/kg)</th>
<th>Dose concentration (mg of oleandrin extract/mL)</th>
<th>Dosing days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Males</td>
<td>Females</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>1.08</td>
<td>4.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>10</td>
<td>1.08</td>
<td>9.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>20</td>
<td>1.08</td>
<td>19.6</td>
</tr>
</tbody>
</table>
Monitoring consisted of mortality checks, clinical observations, body weights, feed consumption, electrocardiograms, and clinical pathology. Cardiology examinations were performed by a board-certified cardiologist pretest (daily for 4 days), predose, and 1 hour postdose and on study day 11 for the male animal. For all available animals, electrocardiogram tracings were recorded 4 times pretest, predose, and 1 hour postdose at each dose level using leads I, II, III, aVR, aVL, and aVF and chart speed of 50 mm/s.

Study II: a 7-day repeat-dose study of oleander extract administered PO to Beagle dogs

A 7-day repeat-dose study of PBI-05204 was conducted in Beagle dogs to determine the relative drug toxicity of oleander in dogs treated with repeated oral doses. A repeat dose of 1 mg/kg of extract (equivalent to 23 μg/kg of oleandrin) daily for 7 continuous days was selected in an attempt to evaluate repeat-dose tolerance to the test article in a relevant species and to cover the expected high dose range for subsequent longer term toxicology studies in Beagle dogs and the proposed clinical study (Table 2). This dose level may produce toxic effects but no excessive lethality that would prevent meaningful evaluation.

Two male and 2 female naïve dogs were administered 1 mg/kg/d of PBI-05204 once daily via oral gavage for 7 consecutive days. The first day of dosing on the study was designated as study day 1. The dose volume for each animal was based on the most recent body weight measurement. Doses were rounded to the nearest 0.1 mL. The doses were given at the same time each day based on study day 1 dosing. Clinical observations, body weight, electrocardiograms, and clinical pathology data were recorded twice daily. Electrocardiograms were performed as noted above. The study was terminated on study day 9, and the animals were released to the methodology colony at the testing facility.

Study III: 28-day toxicity study of oleander extract administered PO to Beagle dogs

The objective of this study was to determine the potential systemic toxicity of test article administered once daily for 28 consecutive days via oral (gavage) administration followed by a 14-day recovery period. A total of 36 dogs were dosed with test article PBI-05204 containing 37.2 mg/mL of oleander extract (oleandrin concentrations of 0.1, 0.3, or 1.0/0.6 mg/kg; groups 2 through 4, respectively), or PBI-05204 control vehicle (placebo; group 1) via oral gavage once daily for 28 consecutive days (except for group 4 animals; Table 3). Due to test article effects (decreased heart rate, cardiac arrhythmias, diarrhea, increased salivation, vomiting, lethargy, and decreased feed), dosing of group 4 animals was suspended for 3 days (study days 10, 11, and 12 in males, and study days 9, 10, and 11 in females) followed by resumption of daily dosing at a reduced dosage level (0.6 mg/kg/d of oleander extract) for the remainder of the study (except for 2 group 4 males). Due to test article effects, 2 group 4 males (animals No. 2951 and 2957), after resumption of dosing on study day 13 at the reduced dosage level, had dosing suspended after study day 13 for the remainder of the study. Three animals of each sex and group were necropsied on study day 29, and the remaining animals were necropsied on study day 42.

Observations for mortality and morbidity were recorded twice daily (AM and PM). Clinical observations were recorded pretest and once daily. Body weights were recorded pretest, on study day 1, and prior to fasting overnight on study day 7. Feed consumption was qualitatively measured daily using terms “normal” (all or the majority of feed eaten), “low” (at least half of the feed remaining), or “scant” (all or the majority of feed remaining) and documented with the clinical observations. General physical examinations were conducted by the staff veterinarian during the pretest period. Physical examinations included a

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of animals</th>
<th>Dose level (mg/kg)</th>
<th>Dose volume (mL/kg)</th>
<th>Dose concentration (mg of oleander extract/mL)</th>
<th>Dosing days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>7</td>
</tr>
</tbody>
</table>

Table 2—Dosing of oleander extract in 4 Beagle dogs in a 7-day repeat-dosing study.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>No. of animals</th>
<th>Dose level (mg/kg)</th>
<th>Dose volume (mL/kg)</th>
<th>Dose concentration (mg of oleander extract/mL)</th>
<th>Dosing days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>Females</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>4</td>
<td>0.1</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>4</td>
<td>0.3</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>5</td>
<td>1.0/0.6*</td>
<td>1.0/0.6*</td>
<td></td>
</tr>
</tbody>
</table>

Table 3—Dosing of 32 Beagle dogs in a 28-day toxicity study.
Results

Study I: a dose range–finding study of oleander extract administered PO to Beagle dogs

Administration of 1 mg/kg/d of PBI-05204 for 7 consecutive days led to development of atrial premature depolarizations and prolongation of atrioventricular conduction (prolonged PR interval and second-degree atrioventricular block). Cardiovascular changes may have contributed to the following clinical findings: abnormal stool and increased salivation on study day 3, vomiting on study day 5, and low feed consumption on study day 7 in males, as well as vomiting on study days 3 and 7 with decreased feed consumption on study day 7 in females. These changes may also contribute to the decrease in reticulocytes and increase in blood urea nitrogen and creatinine (males only) noted on study day 8.
Study III: 28-day toxicity study of oleander extract administered PO to Beagle dogs

In the 28-day toxicity study, administration of test article did not cause a discernible change in QRS duration, QT interval, or QTcf or QTcv throughout the study. However, in dogs receiving 1.0/0.6 mg/kg/d, the heart rate was decreased. In dogs receiving 0.3 or 1.0/0.6 mg/kg/d, atrioventricular conduction was slowed and indicated by prolongation of the PR interval and the development of secondary degree atrioventricular block. Also, atrial premature depolarization developed in dogs receiving over 0.3 or 1.0/0.6 mg/kg/d. The presence of these findings both before and after test article administration in study week 4 suggests at least a 23-hour duration of the test article–induced changes.

There were no deaths during the study. All animals survived until their scheduled necropsy date. Test article–related clinical findings included the following: abnormal stool primarily in group 3 and 4 males, thin body only in group 4 animals with a delayed onset in females when compared to males, decreased activity in group 4 males only, inappetence in group 4 males only, defecation decreased in group 4 males only, predominant postdose vomiting in group 4 (males more than females), predominant increased salivation in group 4 (males more than females), bloodshot eyes in group 4 males only, predominant excessive lacrimation in group 4 animals, and ptosis in group 4 males only. These findings were typically recorded during the dosing period. Compared to study day 1, study day 29 body weights for group 3 and 4 males were decreased. When compared to group 1 controls on study day 29, body weights for group 4 males appear decreased. Similar trends occurred in group 3 and 4 females. There were no apparent test article effects on feed consumption, with the exception of decreased feed intake in group 4 males on study days 7 through 13. There were no apparent test article effects on individual body temperatures, with the exception of decreased body temperatures in group 4 males on study day 8. Based on the measured hematology parameters, there were no apparent biological or toxicological findings. There were no definable test article–related clinical chemistry changes, with the exception of group 4 males on study day 28. Group 4 animal No. 2953, which had recorded macroscopic lung findings and microscopic lung, kidney, liver, and testicular findings, had elevated inorganic phosphorus, decreased sodium, decreased chloride, elevated blood urea nitrogen, and elevated creatinine. Group 4 animals No. 2955 and 2957 had noted increases in ALT levels (increased cell permeability). On study day 29, group 4 male heart weight trends (absolute and relative) were decreased in comparison to concurrent controls. This finding appears to be test article related. There were no abnormal organ weights on study day 42.

In study day 29 animals, dose-dependent gross observations of thin appearance, dark discoloration in the lung, and a dark focus in the lung were considered to be related to the test article and primarily noted for group 4 males (Table 4). These findings were supported by the dose-dependent microscopic findings of acute inflammation, chronic-active inflammation, and hemorrhage in the lung. These findings were considered to be related to the test article; however, it is difficult to exclude the possibility that the test article may have had a synergistic or additive effect related to postdose emesis or reflux followed by test article aspiration. Additional microscopic

Table 4—Summary of test article–related histologic findings in study day 29 animals in the 28-day toxicity study.

<table>
<thead>
<tr>
<th>Finding</th>
<th>Group</th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td>(No. of animals) n = 3</td>
<td>n = 3</td>
</tr>
<tr>
<td>Inflammation, acute</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Minimal</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mild</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Inflammation, chronic active</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mild</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Vacuolation, tubular, epithelium, cortex,</td>
<td></td>
<td>n = 3</td>
<td>n = 3</td>
</tr>
<tr>
<td>bilateral</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mild</td>
<td></td>
<td>n = 3</td>
<td>n = 3</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Degeneration, hydropic, periportal, hepato</td>
<td></td>
<td>n = 3</td>
<td>n = 3</td>
</tr>
<tr>
<td>cellular</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Minimal</td>
<td></td>
<td>n = 3</td>
<td>n = 3</td>
</tr>
<tr>
<td>Testis</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Degeneration, seminiferous tubule, germinal,</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>epithelium, bilateral</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

All other organs examined were normal histologically. NA = Not applicable.
findings included the following: bilateral vacuolation of cortical tubular epithelial cells in the kidney, hydroptic degeneration of periportal hepatocytes in the liver, and bilateral degeneration of the germinal epithelium of the seminiferous tubules in the testis. These findings were considered to be related to the test article and were considered to most likely be secondary to inanition, as the body weight of animal No. 2953 had dropped from 12.9 kg on study day 1 to 9.3 kg on study day 29. There were no macroscopic or microscopic observations in study day 42 animals that were considered to be related to the test article.

Discussion

The results of this study suggest that oleander can be safely administered PO to dogs, but care should be taken to ensure that nontoxic doses are used. A summary of doses and abnormalities noted is included in Supplementary Table S1. At doses ≥ 1 mg/kg/d of oleander extract (equivalent to 23 µg/kg oleandrin), atrial premature depolarizations and prolongation of atrioventricular conduction (prolonged PR interval and second-degree atrioventricular block) were seen. A dose exceeding 20 mg/kg of oleander extract (equivalent to 460 µg of oleandrin/kg) was fatal in 1 dog in the initial study. In dogs receiving 1.0/0.6 mg/kg/d of oleander extract, the heart rate was decreased. In dogs receiving 0.3 or 1.0/0.6 mg/kg/d of oleander extract, atrioventricular conduction was slowed as indicated by prolongation of the PR interval and the development of secondary degree atrioventricular block. Also, atrial premature depolarization developed in dogs receiving over 0.3 or 1.0/0.6 mg/kg/d of oleander extract.

Thus, the studies reported here, taken in totality, suggest that doses exceeding 6.9 µg/kg of oleandrin may be associated with prolongation of the PR interval and development of secondary degree atrioventricular block in dogs. If doses ≥ 1.0 mg/kg/d of oleander extract or 23 µg/kg/d of oleandrin are used, animals should be monitored closely for cardiac arrhythmias. An estimated no treatment effective adverse event oral dose of oleandrin appears to be 4.6 µg of oleandrin/kg. Higher doses may be tolerable but should be used with appropriate monitoring. It should be noted that while effective doses have yet to be fully established in dogs, the authors estimate that effective doses of oleandrin will be approximately ≤ 2 µg/kg/d.

Study limitations included that serum bile acids and plasma and tissue distribution were not evaluated. Further studies are warranted to determine plasma and tissue distribution using safe doses for PO administration of oleandrin noted in this study. Further characterization (eg, Holter monitor) of arrhythmias induced at the higher doses of oleander extract would be of clinical interest.

Acknowledgments

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Disclosures

Dr. Fossum is the CEO of Phoenix Animal Wellness and Dr. Fossum’s Pet Care. The former is a subsidiary of Phoenix Biotechnology Inc, which owns the patents for the oleander extracts used in these studies. Drs. Newman and Matos are members of the Board of Directors for Phoenix Animal Wellness.

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References


