

## 4-Week Toxicity and Toxicokinetic Oral Gavage Study with Polydatin in Rats

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Dr. Lodder was President of Biospherics at the time these data were collected.

# 4-Week Toxicity and Toxicokinetic Oral Gavage Study with Polydatin in Rats

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## Abstract

**Objective:** This study determined the toxicity and toxicokinetics of polydatin when administered via oral gavage to Sprague Dawley rats daily for 4 weeks.

**Background:** Polydatin continues to be investigated as a potential therapy for a variety of human diseases.

**Methods:** Male and female Crl:CD(SD) rats were assigned to five groups, and different doses (0, 300, 600, 1200, or 3000 mg/kg/day) of polydatin were administered via oral gavage once daily for 29 days at a dose volume of 10 mL/kg. Assessment of toxicity was based on mortality, clinical observations, food consumption, body weights, ophthalmic examinations, and clinical and anatomic pathology. Blood samples were collected for toxicokinetic evaluations.

**Results:** All animals survived to the end of the study. Animals given polydatin at 600 mg/kg/day exhibited lower mean body weight (10% compared with control) and less gain in mean body weight, which correlated with decreased food consumption, compared to control rats. Several minor clinical chemistry findings were observed at 600 mg/kg/day, but they were all of small magnitude, and were not considered adverse or toxicologically important. Polydatin related macroscopic findings included one animal with an enlarged cecum in the 1200 mg/kg/day group and 6 of 20 animals with enlarged cecums or colons in the 3000 mg/kg/day group. These findings were considered test article-related, although no microscopic correlate was present. In males given 1200 or 3000 mg/kg/day and females given 3000 mg/kg/day microscopic findings in the kidneys included tubular dilatation, hyaline droplets in the tubule cells (males only), erosion/ulceration of the transitional epithelium, acute pelvic inflammation (males only), and chronic active pelvic inflammation with hyperplasia of the transitional epithelium (females only). No marked sex differences were observed in polydatin  $C_{max}$  and  $AUC_{0-24}$  values. No accumulation of polydatin was observed after multiple dosing.

**Conclusion:** The only adverse test article-related finding noted in one female given 3000 mg/kg/day was chronic active pelvic inflammation and transitional cell hyperplasia. Based on these results, the no observed

adverse effect level (NOAEL) is 1200 mg/kg/day for females and 3000 mg/kg/day for males.

## Introduction

There is considerable interest in the use of polydatin for the treatment of a variety of human diseases [1]. Polydatin ((PD) also known as trans-polydatin, piceid, and 3,4',5-trihydroxystilbene-3- $\beta$ -mono-D-glucoside) is a glucoside derivative of resveratrol. It is purified from the root of *Polygonum cuspidatum* but can also be found in wines and grapes [2-5], cocoa [6], peanuts and peanut butter [7], pistachios [8] and almonds [9]. Extracts derived from *Polygonum cuspidatum* have long been a part of traditional Chinese herbal medicine being used to treat pain, fever, coughs, inflammation and a variety of other ailments [10]. Polydatin is found to be the major component of these extracts. As a derivative of resveratrol, it is believed to have many of the same beneficial effects but has some properties that may make it more effective from a pharmacological standpoint than resveratrol. Polydatin is structurally the same as resveratrol except that it has a glucoside group attached to the C-3 position in place of a hydroxyl group. This substitution makes polydatin more water soluble and resistant to enzymatic breakdown than resveratrol [11]. It is also actively taken up by cells via glucose carriers in the cell membrane instead of being passively transported like resveratrol [12, 13]. These properties suggest that polydatin may have greater bioavailability than resveratrol.

Studies have presented evidence that polydatin has many positive health effects. These effects include anti-inflammatory [14, 15], hepatoprotective [16-19], anti-cancer [20-23], neuroprotective [14, 24-26], and cardioprotective activities [27-31]. Additional studies demonstrated that polydatin also has protective effects against shock [32-34], ischemia/reperfusion injury [35,36], congestive heart failure [37], endometriosis [38], prevents fatty liver disease and insulin resistance [39], and that it can regulate glucose and lipid metabolism [40]. Polydatin has been studied in clinical trials for the treatment of hemorrhagic shock and irritable bowel syndrome [41, 15].

The use of polydatin as a potential therapy for dyslipidemia has been suggested by studies using

animal models. Arichi et al. [42] discovered that orally administered polydatin (100 mg/kg body weight) significantly lowered low-density lipoprotein (LDL)-derived cholesterol by approximately 18% and serum triglycerides by 40% in rats consuming standard chow containing a mixture of corn oil, 10% cholesterol, and 1% cholic acid. Although lower doses of trans-polydatin (50 mg/kg body weight) were ineffective at preventing hyperlipidemia, they were able to prevent the accumulation of cholesterol and triglycerides in the liver, suggesting that lower doses may also be effective but to a much lesser extent. In a study using Syrian golden hamsters, polydatin was found to decrease total cholesterol levels and total triglyceride levels by 47% and 63%, respectively, compared to standard diet [28]. In another study using rabbits, the administration of polydatin decreased the serum levels of total cholesterol, triglycerides and LDL [29]. The ratio of total cholesterol to HDL was also reduced.

Insulin, through activation of the Akt pathway and other metabolic pathways, is a major component of metabolic regulation [43]. Hao et al. [40] recently found that polydatin activated the Akt signaling pathway in diabetic rats, possibly by phosphorylation of the insulin receptor substrate (IRS), thus reducing blood glucose levels. Polydatin may also decrease the expression of intercellular adhesion molecule 1 (ICAM-1) and may reduce white blood cell adhesion, as well as the effects of other cell adhesion molecules and inflammatory cytokines, thought to be active in early atherosclerotic development [11]. Additionally, polydatin is also thought to provide protection from oxidative peroxidation, which can result in cell damage [12-44], and inhibit oxidation of LDL particles which may also play a role in atherosclerosis [32].

Both human and animal studies suggest that orally administered polydatin is absorbed in minutes, metabolized in minutes to hours, and eliminated in 24 hr [46-48]. Metabolites of trans-polydatin include glucuronidated and/or sulfonated trans-polydatin, trans-resveratrol, and glucuronidated and/or sulfonated trans-resveratrol.

This study determined the toxicity and toxicokinetics of polydatin when administered via oral gavage to Sprague Dawley rats daily for 4 weeks.

## Methods

### Regulatory Guidelines

The study was conducted according to Good Laboratory Practice (GLP) and based on the principles

of the Food and Drug Administration Center for Drug Evaluation and Research (CDER)/International Conference on Harmonisation (ICH) Harmonised Tripartite Guidelines ICH-M3, Nonclinical Safety Studies for the conduct of Human Clinical Trials for Pharmaceuticals (CDER, July 1997) and S3A, Toxicokinetics: The Assessment of Systemic Exposure in Toxicity Studies (CDER, March 1995).

### Animals

Male and female Crl:CD(SD) rats were received from Charles River Laboratories, Portage, Michigan. At initiation of dosing, the animals were 6 to 7 weeks old, and their body weights ranged from 238 to 303 g for males and 143 to 238 g for females. Animals were randomized to the study groups using a computerized procedure designed to achieve body weight balance with respect to subgroup assignment, within a 5% probability of homogeneity of variance. Following randomization, each study animal was assigned a unique number by means of an implantable microchip identification device and/or cage card. The oral route of administration was selected because it is the intended route of administration in humans.

Male and female rats were housed individually in stainless steel cages in the following conditions: temperature range of 20 to 26°C, relative humidity range of 30 to 70%, 10 or greater air changes/hour, and a 12-hour light/12-hour dark cycle. The light/dark cycle was interrupted for study-related activities.

### Diets and Test Materials

Animals were offered Certified Rodent Diet #2016C (Harlan Laboratories, Inc.) ad libitum unless fasted for study procedures. Water was provided ad libitum.

### Experimental Design

Testing was performed by Covance Laboratories (Madison, Wisconsin). The test article, polydatin, was supplied by Biotivia (purity 98.36% by HPLC, Lot. No. BIPL110714) and was stored at 2 to 8 °C. The vehicle control article was reverse osmosis water. Test article formulations were prepared daily Days 1 through 5 of the dosing phase and once weekly from day 6 through the remainder of the dosing phase. Formulations prepared prior to the day of dosing were stored in a refrigerator, set to maintain 2 to 8°C, and protected from light until removed for dosing. Formulations prepared on the day of dosing were stirred continuously using a magnetic stir bar and stir plate and stored protected from light at room temperature. Formulations were kept at approximate room temperature no longer than 8 hours.

Animals were weighed 3 days prior to receiving their

first dose, on the day they received their first dose, and weekly thereafter. All animals were checked twice daily (a.m. and p.m.) for mortality, abnormalities, and signs of pain or distress. At a minimum, clinical observations made from cage side were recorded daily during the dosing phase. Detailed clinical observations were recorded on toxicity animals once during the predose phase, prior to dosing on Day 1, and weekly (based on Day 1) throughout the dosing phase, and the termination date. Food consumption was measured for the toxicity groups. Ophthalmic evaluations were performed once during prephase and on Day 29 of the dosing phase by a qualified veterinarian.

Polydatin was administered by oral gavage once daily for 29 days (dosing phase) at a dose volume of 10 mL/kg. according to Illustration 1 Study Groups. Doses were stirred for at least one hour prior to and during the dosing. The dose levels selected were based on available data from the literature of toxicity of resveratrol [49] in addition to studies evaluating efficacy of polydatin [50-52]. Statistical power analysis suggests 30 mg/kg is the minimum dose capable of causing a statistically significant reduction in cholesterol and triglycerides based on results in the  $LDLr^{-/}$  mouse (Unpublished study no.70971-0005). The dose level of 300 mg/kg is an order of magnitude larger and is the dose for which there are published kinetic data in the literature [47, 53]. The upper limit of 3000 mg/kg is another order of magnitude larger and a level at which some effects on the kidney have been reported [54].

After 29 days of dosing, all surviving toxicity animals were sacrificed and necropsied. Terminal body weights were recorded. Animals were anesthetized with sodium pentobarbital and exsanguinated. An examination of the external features of the carcass, external body orifices, abdominal, thoracic, and cranial cavities, organs, and tissues were performed. A pathologist was available for consultation.

Organ weights, including adrenal (2), brain, epididymis (2), heart, kidney (2), liver, lung, ovary (2) pituitary gland, prostate, salivary gland, mandibular (2), seminal vesicle, spleen, testis (2), thymus, thyroid (2 lobes) with parathyroid, and uterus, were recorded for toxicity animals. Paired organs were weighed together. Adrenal (2), aorta, brain, cecum, cervix, colon, duodenum, epididymis (2), esophagus, eye (2), a femur with bone marrow (articular surface of the distal end), Harderian gland, heart, ileum, muscle (biceps femoris), optic nerve (2), ovary (2), pancreas, pituitary gland, prostate, rectum, salivary gland [mandibular (2)], sciatic nerve, seminal vesicle, skin/ subcutis, spinal

cord (cervical, thoracic, and lumbar), spleen, sternum with bone marrow, stomach, jejunum, kidney (2), lesions, liver, lung with large bronchi, lymph node (mandibular), lymph node (mesenteric), mammary gland (females), testis (2), thymus, thyroid (2 lobes) with parathyroid, tongue, trachea, urinary bladder, uterus, and vaginal tissues (when present) from each toxicity animal were preserved in 10% neutral buffered formalin, with the exception of the eyes, Harderian gland, optic nerves, and testes, which were collected in modified Davidson's fixative and stored in 10% neutral-buffered formalin.

For histopathology, tissues were embedded in paraffin, processed to slide, and stained with hematoxylin and eosin. All preserved tissues (see above) were examined for histopathologic irregularities from toxicity animals in the control and high-dose groups and kidney, liver, and macroscopic lesions from all animals in the low-, mid-, and mid-high dose groups were examined microscopically by a veterinary pathologist. In addition, based on microscopic findings in the control and high dose groups, the urinary bladders from 2 females in Group 3, Subgroup1 and the spleen from males in the low-, mid-, and mid-high dose groups were examined microscopically by the same veterinary pathologist. Additionally, any macroscopic lesions or suspected target organs of note at the high dose range, were examined.

Blood samples were collected from toxicity animals (see Illustration 1) for hematology, clinical chemistry, and coagulation via a jugular vein from animals fasted overnight. Samples were collected on the day of scheduled sacrifice. Sodium citrate and potassium EDTA were used as anticoagulants for coagulation and hematology tests, respectively. Samples for clinical chemistry were collected without anticoagulant. Urine samples for urinalysis were collected chilled on wet ice during the overnight period from fasted toxicity animals. Samples were collected on the day of scheduled termination.

Blood samples (approximately 0.25 mL) were collected prior to dosing via a jugular vein from toxicokinetic animals (Group 2, Subgroup 2, nine animals/sex, see Illustration 1) given 300 mg/kg/day predose (Day 25 only). Nine animals per sex were used with blood samples collected from three animals/sex/time point. Blood samples were collected from three animals/sex/timepoint on Days 1 and 25 at the following time points: predose (Day 25 only) and at approximately 0.167, 0.333, 0.5, 1, 2, 4, 8, 12, and 24 hours postdose. Samples were collected in tubes containing potassium EDTA and maintained on chilled cryoracks until centrifugation. Samples were

centrifuged within 1 hour of collection, and plasma was harvested. Following centrifugation, samples were processed under yellow light. Plasma samples were stored in a freezer, set to maintain -60 to -80°C, until analyzed. Plasma analysis for *trans*-polydatin (also known as polydatin) and *trans*-resveratrol was performed by Covance-Madison using a method (Method SPXRPP) previously validated under Covance Study No. 8251389. Incurred sample reproducibility was conducted in accordance with Covance standard operating procedures.

Toxicokinetic analysis included (when appropriate), but was not limited to, maximum observed concentration (C<sub>max</sub>), time to peak concentration (T<sub>max</sub>), and area under the concentration-time curve (AUC). Area under the concentration-time curve from hour 0 to infinity for Day 1 calculated as follows:  $AUC_{0-\infty} = AUC_{0-t} + C_t/\lambda_z$ . Where C<sub>t</sub> is the last measurable concentration and λ<sub>z</sub> is the elimination rate constant estimated using log-linear regression during the terminal elimination phase. The number of points used in λ<sub>z</sub> calculation was determined by visual inspection of the data describing the terminal phase. At least the last three time points with measurable values were used in λ<sub>z</sub> calculation.

#### Statistical Analysis

Data for each sex were analyzed separately; only data collected on or after the first day of dosing were analyzed statistically. Only data from toxicity animals (Subgroup 1) were evaluated. Analysis of variance (ANOVA) [55] and pairwise comparisons were used to analyze the following; absolute body weight, body weight change, quantitative food consumption, continuous clinical pathology values, terminal body weight, absolute organ weight, organ to body weight percentage, and organ to brain weight percentage.

Levene's test [56, 57] was done to test for equality of variances between groups. Where Levene's test was significant (p < 0.05), a rank transformation (to stabilize the variances) was applied before the ANOVA was conducted (note: Levene's test was not applied to the rank-transformed data). Where Levene's test was not significant (p > 0.05), ANOVA was conducted.

One-way ANOVA was used (if applicable) to analyze continuous clinical pathology values, absolute organ weight data, food consumption, and body weight data. If the group effect of the ANOVA was significant (p < 0.05), Dunnett's t-test [58, 59] was used for pairwise comparisons between each treated and control groups. Group comparisons (Groups 2 through 5 versus Group 1) were evaluated at the 5.0%,

two-tailed probability level.

## Results

### Body Weights

Animals given 600, 1200, or 3000 mg polydatin/kg/day showed lower mean body weights that were dose dependent (Illustration 2). Decreases were of small magnitude (~10% compared with control) and correlated with lower body weight gain in these animals (Illustration 3). Statistically significant decreases included lower mean body weight for all 4 weeks of the dosing phase for males given 3000 mg/kg/day. While the decreases were not always statistically significant, dose dependency and a correlating decrease in food consumption suggested decreases in body weight and body weight gain were polydatin related. Due to the absence of relevant clinical observations and small magnitudes of the changes, decreases in body weight and body weight gain were not considered adverse. In addition, polydatin is a prodrug of resveratrol, and resveratrol is a caloric restriction mimetic [60, 61]. For this reason, a dose-dependent decrease in body mass is not surprising (See Illustration 4).

### Food Consumption

Males and females given 3000 mg polydatin/kg/day consumed less food during Week 1 of the dosing phase (Illustration 5). This decrease was statistically significant for males. Lower food consumption continued to be observed for males and females for Weeks 2 and 3 of the dosing phase; however, it was not statistically significant and the difference became smaller in magnitude with each week. During Week 4 of the dosing phase, food consumption for males and females given 3000 mg/kg/day was comparable with control rats. Lower food consumption was also observed during Weeks 1, 2, 3, and 4 (females only) of the dosing phase for animals given 600 or 1200 mg/kg/day (Illustration 5). However, this was not statistically significant. This decrease was considered polydatin-related given that a correlating decrease in body weight was observed for these animals. Due to the absence of relevant clinical observations and small magnitudes of the changes, decreases in food consumption were not considered adverse.

### Toxicokinetic Analyses

#### *Polydatin*

Mean concentration time profiles for males and females showed mean concentrations of polydatin were generally similar after a single dose and multiple

doses of polydatin (Illustration 6). After oral administration, polydatin was readily absorbed, with  $T_{max}$  values ranging from 0.500 to 1.00 hours on Days 1 and 25 of the dosing phase. Polydatin was readily eliminated, and concentration values for polydatin were below the limit of quantitation by 12 hours postdose on Days 1 and 25 of the dosing phase.

$C_{max}$  and  $AUC_{0-24}$  values for polydatin were higher for females than for males, but the differences were less than 2-fold. Values for  $C_{max}$  and  $AUC_{0-24}$  were lower on Day 25 compared with Day 1 of the dosing phase, indicating no accumulation of polydatin after multiple dosing.

#### *Trans-Resveratrol*

Mean concentration time profiles for males and females showed mean concentrations of *trans*-resveratrol were generally similar after a single dose and multiple doses of polydatin (Illustration 6). After oral administration, *trans*-resveratrol readily appeared in the plasma, with  $T_{max}$  values of 2.00 hours on Days 1 and 25 of the dosing phase. Plasma levels readily declined and were below the limit of quantitation by 24 hours post-dose on Days 1 and 25 of the dosing phase.  $C_{max}$  and  $AUC_{0-24}$  values for *trans*-resveratrol were higher for males than for females, but the differences were less than 2-fold. Values for  $C_{max}$  and  $AUC_{0-24}$  were lower on Day 25 compared with Day 1 of the dosing phase, indicating no accumulation of polydatin after multiple dosing. Mean  $AUC_{0-24}$  ratios of *trans*-resveratrol to polydatin (metabolite-to-parent ratio) were 0.352 and 0.358 for males and 0.195 and 0.190 for females on Days 1 and 25 of the dosing phase, respectively.

Although maximum serum levels of polydatin were slightly less in males than in females, both sexes absorbed polydatin with similar kinetics, reaching peak levels (approximately 3.0 mg/ml) within 1 hr of dosing (Illustration 7). Serum *trans*-resveratrol levels were 10-fold less than polydatin and attained maximal levels in both male and females approximately 2 hr after dosing. Importantly, the levels of both polydatin and *trans*-resveratrol were below the limit of detection 24 hr after dosing. Similar results were also found on day 25 of the toxicokinetic study indicating that there was no accumulation of polydatin or resveratrol after repeat dosing.

#### Clinical Pathology, Hematology and Coagulation

No polydatin-related effects were observed in hematology and coagulation data.

Minimally shortened prothrombin time in males given  $\geq 1200$  mg/kg/day was of small magnitude ( $\leq 0.5$  sec) and, considering the normal variability in coagulation

times, was not considered test article-related or toxicologically important. Shortened prothrombin time was slightly surprising as others have found polydatin protects against thrombosis possibly by decreasing platelet-neutrophil interactions [62].

#### Clinical Chemistry

Several minor polydatin-related findings were observed in clinical chemistry test [63] results but they were of small magnitude and not considered adverse or toxicologically important. Findings at  $\geq 600$  mg/kg/day included the following.

Minimally lower glucose in males given  $\geq 600$  mg/kg/day and females (not statistically significant) given 3000 mg/kg/day

Minimally higher albumin in males given  $\geq 1200$  mg/kg/day

Minimally lower globulin and higher albumin-to-globulin ratio in males given  $\geq 600$  mg/kg/day

Minimally higher ALT activity in males and females given  $\geq 600$  mg/kg/day

Minimally higher alkaline phosphatase (ALP) activity in females given  $\geq 600$  mg/kg/day

Minimally lower potassium in females given 3000 mg/kg/day

Minimally higher alanine aminotransferase (ALT) activity in males was the only clinical chemistry finding at 300 mg/kg/day. Overall, elevations in ALT (1.2 to 2.1x control) and ALP (1.3 to 1.6x control) were of small magnitude and not associated with microscopic findings in the liver. No rise was detected in total bilirubin, also a strong indicator of drug induced liver injury (DILI). Other studies have shown polydatin to have a protective hepatic mechanism in mice dosed up to 300mg/kg [64] and 100 mg/kg [19].

Lower glucose (See Illustration 8) and potassium may have been associated with reduced feed consumption observed in these animals. However, as we discussed earlier, previous studies have identified polydatin as capable of lowering glucose levels in diabetic models of mice [40] and rats [11] by stimulating insulin secretion. High albumin (See Illustration 9) commonly occurs with dehydration, however, dehydration was not evident clinically or in other clinical pathology data (serum urea nitrogen and creatinine). It should be noted that lower albumin levels are more often associated with liver disease [65], nephrotic syndrome, [66] and malnutrition [67].

#### Urinalysis

None of the urinalysis findings were considered

adverse or toxicologically important. Minimally lower urine pH in males and females given  $\geq 1200$  mg/kg/day and higher incidence or severity of urine occult blood and presence of red blood cells in the urine of a few males given 3000 mg/kg/day and presence of white blood cells in the urine of a few females given 3000 mg/kg/day were considered polydatin-related findings but not adverse. The change in pH appeared consistently present in most animals, but the remaining urinalysis findings were observed in individual animals and most were not associated with correlative microscopic findings.

One female given 3000 mg/kg/day had increased severity of white blood cell in the urine, which correlated with marked chronic active pelvic inflammation observed microscopically in the kidneys (See Illustration 10). However, one male given 1200 mg/kg/day with microscopic evidence of marked acute renal hemorrhage had no occult blood or presence of red blood cells in the urine. Additionally, two females given 600 mg/kg/day with microscopic findings of chronic active inflammation in renal pelvis (see Illustration 10) had no increase in evidence or severity of white blood cells in the urine, although another animal had presence of red blood cells in the urine.

Test results of interest included increased incidence and severity of tubular dilatation (see Illustration 11), increased incidence and severity of hyaline (protein) droplets in the tubule cells (males only) (see Illustration 12), erosion/ulceration of the transitional epithelium (see Illustration 13), and inflammation of the renal pelvis (see Illustration 10).

Statistically significant or otherwise notable differences observed for other clinical pathology test results were considered incidental because they were usually of very small magnitude and/or lacked a relationship to dose.

#### Anatomic Pathology

Polydatin related terminal body weight changes were noted in animals given 3000 mg/kg/day compared with controls. Mean terminal body weight was 10% lower in males and 8% lower in females; the change was only significant in males. Brain-to-body weight and kidney-to-body weight ratios were significantly higher in males given 3000 mg/kg/day, which were attributed to the lower terminal body weight. Absolute liver weight in males given 3000 mg/kg/day was significantly lower (12.9%) than controls; these findings were considered spurious since no microscopic correlate was present.

Absolute and relative mean spleen and thymus weights were also lower in males and females

given 3000 mg/kg/day, though not significantly. There was no microscopic correlate for the spleen or thymus weight changes; they were attributed to biological variation. Individual spleen weights did not correlate with the lymphocyte depletion noted below.

Macroscopically, at 3000 mg/kg/day, large cecum was noted in 2/10 males and 3/10 females, and a large colon was noted in 1/10 males. At 1200 mg/kg/day, a large cecum was noted in 1/10 females. This finding was considered test article-related, although no microscopic correlate was present.

Polydatin-related microscopic kidney findings were limited to males given  $\geq 1200$  mg/kg/day and females given 600 or 3000 mg/kg/day. Kidney findings were sporadic, varied between animals, and did not often show a clear dose response relationship. The only microscopic finding that was considered adverse was the marked bilateral chronic active inflammation and transitional cell hyperplasia in one female given 3000 mg/kg/day (See Illustration 10). Although chronic inflammation was only noted in the aforementioned animal, it was considered test article-related because of the presence of minimal to slight acute or chronic/active inflammation in several other treated animals given lower doses, the marked severity of the lesion, and the lack of evidence of any other inciting cause (i.e., a calculus causing chronic irritation). Similar inflammation in two females given 600 mg/kg/day was not considered adverse due to the lower severity and the unilateral nature of the inflammation. The hyaline protein droplets in the tubular cells of the males were also not considered adverse, even at a marked severity, because there was no clinical pathology evidence of renal protein loss.

An unusual finding of uncertain relationship to the test article was the presence of marked acute hemorrhage in the kidney of one male given 1200 mg/kg/day. The cause of the hemorrhage was not evident in examined sections. This animal also was noted to have a urinary bladder that was discolored and filled with red fluid at necropsy.

Lymphocyte depletion was observed in the mantle zone of the spleens in 4/10 males in the test article group given 3000 mg/kg/day (See Illustration 14). This finding was not considered a primary test article related effect, and was attributed to stress. Lymphocyte depletion was not seen in any other examined lymphoid organs (thymus, mesenteric lymph node, mandibular lymph node, and gastrointestinal lymphoid tissue present on examined sections of intestine).

## Discussion

In this study, administration of polydatin up to 3000 mg/kg/day did not result in any adverse effects on the general health of the animals. Although administration of polydatin caused reductions in body weight, body weight gain, and food consumption, decreases in body weight and food consumption were of small magnitude. Furthermore, decreases in food consumption were primarily noted during the first 3 weeks of dosing, suggesting animals were starting to adapt by Week 4 of the dosing phase

Reduction in lymphocytes appears to be systemic and likely related to stress. One cortisol treatment has been shown to reduce lymphocytes by as much as 70% in humans [68]. Other occurrences in the study which could be interpreted as stress-related include changes in the spleen cellularity and lymph node cellularity in males, and increases in circulating neutrophils compared to control (although not significant) [69].

Other test related findings included minimally higher ALT in females dosed at 2600 mg/kg/day but there was no increase in liver weight, or rate of hepatocellular changes denoting necrosis or degeneration of the bile duct greater than seen in the control group. Similar studies in humans have been reported in which weight loss in women was associated with an increase in ALT and AST but no hepatic histological changes were noted [70].

Urinary related findings included dilated tubules, presence of hyaline droplets, dilation of the renal pelvis, inflammation of the pelvis, and hyperplasia and or ulceration of the transitional epithelium. Dilated tubules may be regarded as normal if present in small amounts [71]. Hyaline droplets are also of little concern if restricted to younger male animals as seen in the study and appear in equal rates in the treated and control groups [72]. The only adverse test article-related finding noted in one female given 3000 mg/kg/day was chronic active pelvic inflammation and transitional cell hyperplasia. Based on these results, the no observed adverse effect level (NOAEL) is 1200 mg/kg/day for females and 3000 mg/kg/day for males.

All animals in the toxicokinetic phase of the study (300 mg/kg/day) were exposed to polydatin and its metabolite, *trans*-resveratrol as verified by the presence of these two materials in the plasma samples. No marked sex differences were observed in polydatin and *trans*-resveratrol  $C_{max}$  and  $AUC_{0-24}$  values. No accumulation of polydatin or *trans*-

resveratrol was observed after multiple dosing of polydatin.

## Conclusion

All animals survived to the end of the study. No polydatin-related clinical observations or ophthalmic findings were noted. No marked sex differences were observed in polydatin  $C_{max}$  and  $AUC_{0-24}$  values. No accumulation of polydatin was observed after multiple dosing. The only adverse test article-related finding noted in one female given 3000 mg/kg/day was chronic active pelvic inflammation and transitional cell hyperplasia. Based on these results, the no observed adverse effect level (NOAEL) is 1200 mg/kg/day for females and 3000 mg/kg/day for males.

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## Illustrations

### Illustration 1

Illustration 1

Illustration 1.

Study Groups.

Group	Subgroup	No. of Animals		Dose Level (mg/kg/day)	Dose Concentration (mg/mL)
		Male	Female		
1 (Control) <sup>a</sup>	1 (Toxicity)	10	10	0	
2 (Low)	1 (Toxicity)	10	10	300	30
	2 (Toxicokinetic)	9	9	300	30
3 (Mid)	1 (Toxicity)	10	10	600	60
4 (Mid-High)	1 (Toxicity)	10	10	1200	120
5 (High)	1 (Toxicity)	10	10	3000	300

<sup>a</sup> Group 1 received vehicle control article only.

### Illustration 2

Illustration 2

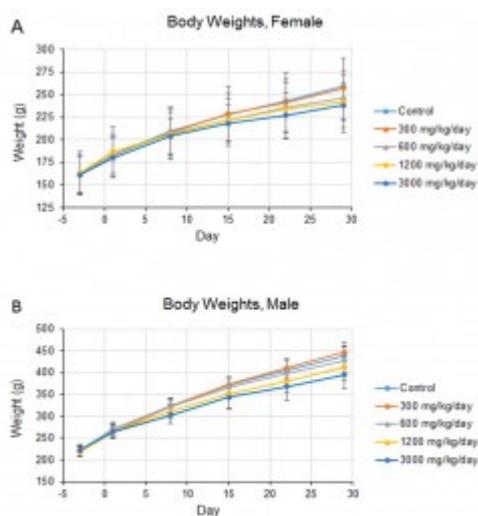


Illustration 2. Weight gain of rats on different doses of polydatin over the course of the experiment. Rats received their first dose of polydatin on Day 1. (A) Female rats, n=10 per group. (B) Male rats, n=10 per group. Results are shown as mean  $\pm$  stdev.

### Illustration 3

Illustration 3

Illustration 3

Percent Decrease in Body Weight

Body Weight (% lower compared with control)									
Group	Dose (mg/kg/day)	Males				Females			
		Day 8	Day 15	Day 22	Day 29	Day 8	Day 15	Day 22	Day 29
2	300	-	-	-	-	-	-	-	-
3	600	-	1.6	1.5	2.5	-	2.6	2.9	5.0
4	1200	4.3	5.3	5.9	5.9	1.4	2.6	3.7	6.6
5	3000	6.8*	7.5*	9.1*	10.0*	2.4	4.4	6.6	8.1

- = No noteworthy difference. \* = Statistically significant at P <= 0.05.  
 Body weight for animals given 300 mg/kg/day were comparable with controls.

### Illustration 4

Illustration 4

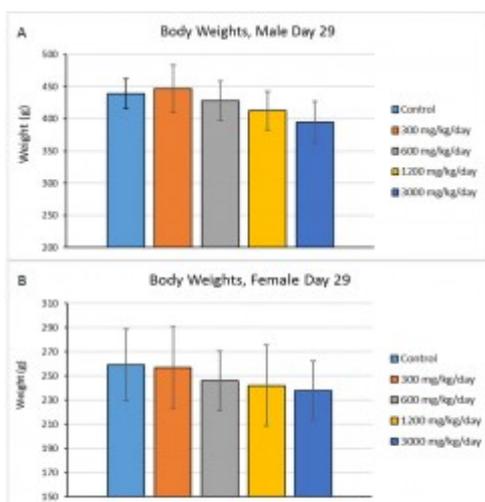


Illustration 4. Body weights on Day 29 prior to study termination. A) Female rats, n=10 per group; B) Male rats, n=10 per group. \* P<0.05 compared to control. Results are shown as mean ± stdev.

## Illustration 5

Illustration 5

Illustration 5

Percent Decrease in Food Consumption  
Food Consumption (% lower compared with control)

Group	Dose (mg/kg/day)	Males				Females			
		Week	Week	Week	Week	Week	Week	Week	Week
		1	2	3	4	1	2	3	4
1	0	-	-	-	-	-	-	-	-
2	300	-	-	-	-	-	-	-	-
3	600	2.3	3.6	2.2	1.9	1.4	2.0	3.0	4.5
4	1200	6.5	3.6	3.5	-	6.8	7.9	7.8	5.7
5	3000	11.1*	6.7	4.4	-	10.2	7.9	6.6	3.2

- = No noteworthy difference. \* = Statistically significant at P <= 0.05.

## Illustration 6

Illustration 6

Illustration 6

Toxicokinetic Parameters for Polydatin and Trans-Resveratrol in Rat Plasma

Analyte	polydatin Dose Level (mg/kg/day)	Day	Sex	C <sub>Tmax</sub> (ng/mL)	T <sub>Tmax</sub> (hr)	AUC <sub>0-4</sub> (ng·hr/mL)	AUC <sub>0-24</sub> (ng·hr/mL)	AUC <sub>0-∞</sub> (ng·hr/mL)	M/P AUC <sub>0,24</sub> Ratio
polydatin	300	1	M	2857	1.00	9699	10291	NC	
			F	3183	0.500	10640	10964	11003	
		25	M	2690	1.00	7629	7741	NA	
			F	4700	0.500	9472	9593	NA	
trans- resveratrol	300	1	M	467	2.00	3235	3619	NC	0.352
			F	333	2.00	2064	2139	NC	0.195
		25	M	302	2.00	2171	2773	NA	0.358
			F	292	0.500	1630	1827	NA	0.190

NA = Not applicable; NC = Not calculated.

## Illustration 7

Illustration 7

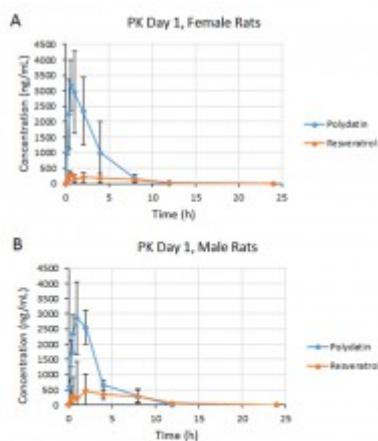


Illustration 7. Serum concentrations of polydatin and trans-resveratrol after the oral gavage of 0.3 g/kg of polydatin in female (A) and male (B) rats. Nine animals per sex were used with blood samples collected from three animals/sex/timepoint. Graphs represent the results obtained on the first day of dosing. The levels of polydatin and trans-resveratrol were assumed to be 0 at time zero because the rats had not been previously exposed to polydatin. Results are shown as mean ± standard deviation.

## Illustration 8

Illustration 8

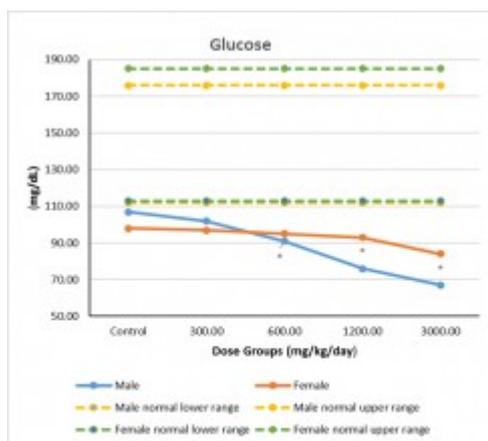


Illustration 8. Glucose serum levels of male and female rats on Day 29. Female rats, n=10 per group; Male rats, n=10 per group.

\*= Statistically significant at  $P \leq 0.05$ .

Normal value range based on Charles River historical data of CrI:CD(SD) Rats [73]

## Illustration 9

Illustration 9

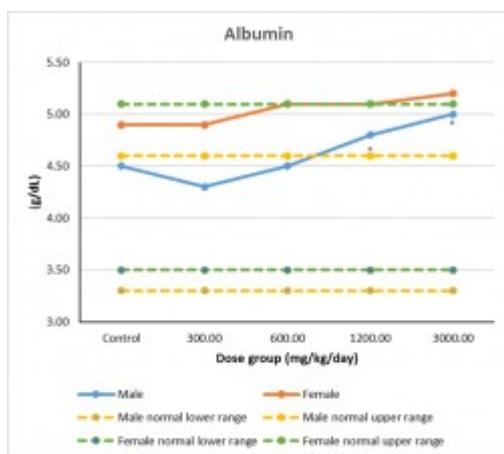


Illustration 9. Albumin serum levels of male and female rats on Day 29. Female rats, n=10 per group; Male rats, n=10 per group.

\*= Statistically significant at P <= 0.05.

Normal value range based on Charles River historical data of CrI: CD (SD) Rats [73]

## Illustration 10

Illustration 10

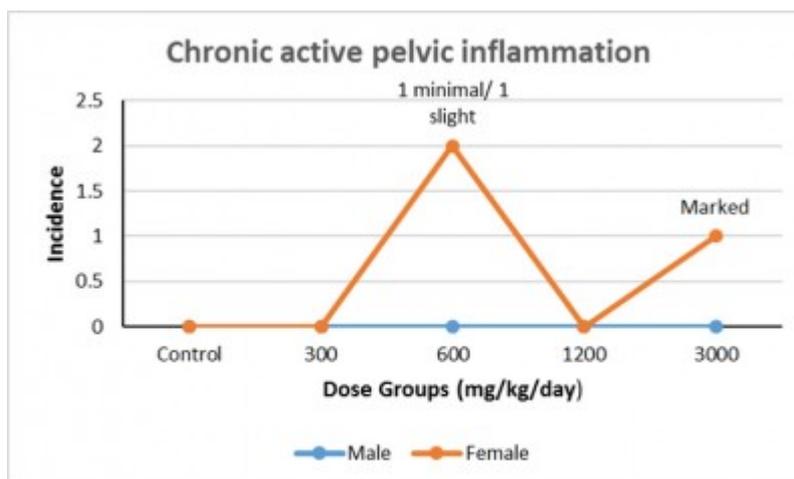


Illustration 10. Chronic active pelvic inflammation in male and female rats. Female rats, n=10 per group; Male rats, n=10 per group.

## Illustration 11

Illustration 11

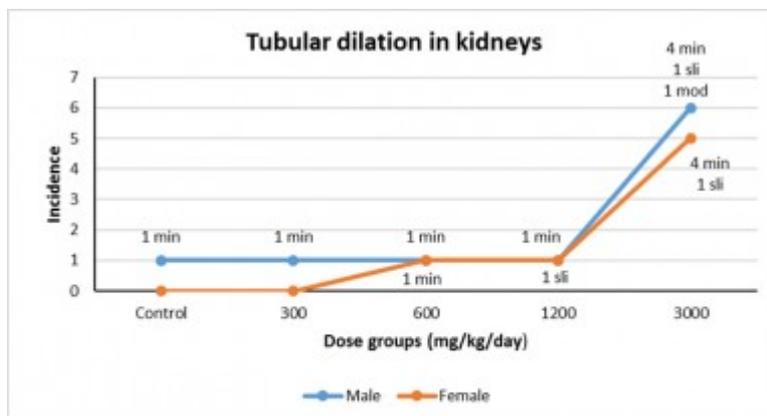


Illustration 11. Tubular dilation in kidneys in male and female rats. Female rats, n=10 per group; Male rats, n=10 per group. Abbreviations: min-minimal; sli-slight; mod-moderate

## Illustration 12

Illustration 12

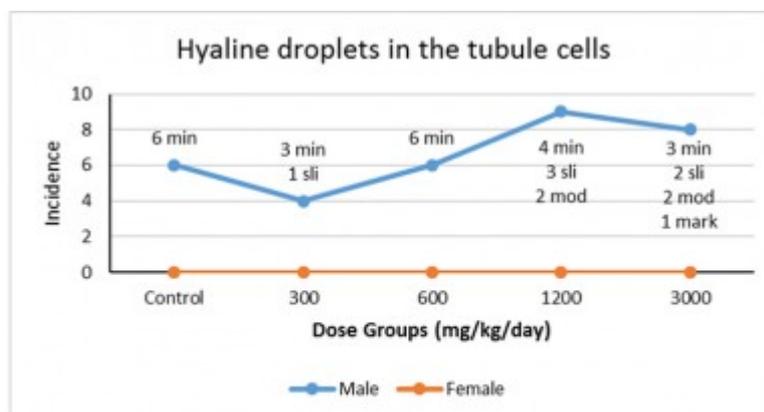


Illustration 12. Hyaline droplets in the tubule cells of male and female rats. Female rats, n=10 per group; Male rats, n=10 per group. Abbreviations: min-minimal; sli-slight; mod-moderate; mark-marked

## Illustration 13

Illustration 13

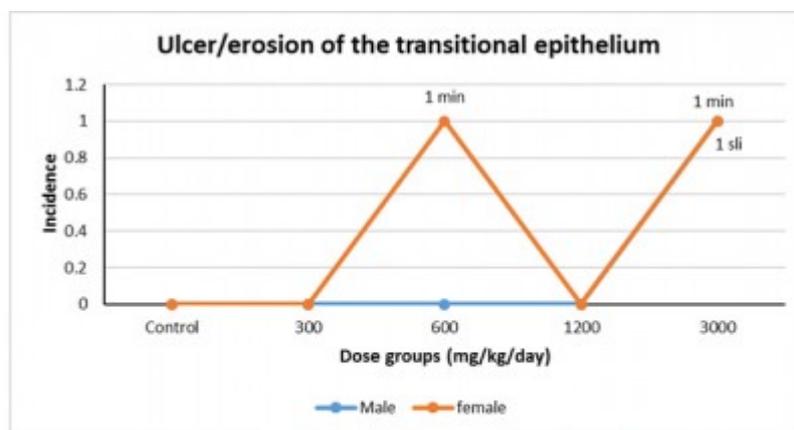


Illustration 13. Ulceration of the transitional epithelium in male and female rats. Female rats, n=10 per group; Male rats, n=10 per group.

Abbreviations: *min*-minimal; *sli*-slight

## Illustration 14

Illustration 14

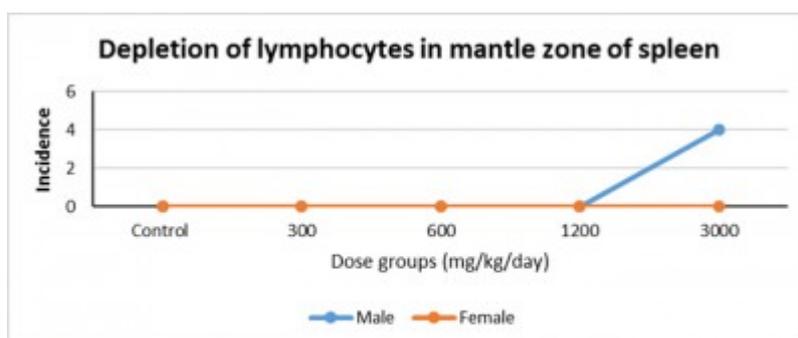


Illustration 14. Depletion of lymphocytes in mantle zone of spleen in male and female rats. Female rats, n=10 per group; Male rats, n=10 per group.