Acute toxicity potential and impact on periodontal and periapical tissue of Pulp Out: A paste contained jatropha, sidaguri, and melittin

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Pulp Out, paste-contained jatropha sap, sidaguri roots, and melittin, has been studied to have potency used as an herbal-based devitalizing agent. Prior to clinical application, the toxicity of Pulp Out should be evaluated as it might leak from the cavity unintentionally and get into the digestive systems, which can cause either local or systemic effects. The present study aimed to evaluate the impact of Pulp Out application on periodontal and periapical tissue as well as acute toxicity in Wistar rats. The paste was inserted into the periapical tissue. After 7 days, the periapical tissue was isolated for histopathological evaluation. Pulp Out in an oral suspension of 50, 500 and 2500 mg/kg BW was administered. Autonomic nerve signs were observed intensively every 4 h as well as water and food consumption for 14 days. Biochemical, hematologic parameters and specific organs were evaluated. Therefore, considering the inflammatory lymphocyte cells, osteoblasts, and osteoclasts, Pulp Out is not toxic. The acute toxicity study revealed no treatment-related death was observed, indicating that LD50 is greater than 2500 mg/kg BW. No significant difference statistically either in body weight, water or food consumption as was observed in autonomic clinical signs of treated groups when compared to the control. Similarly, biochemical and hematologic properties showed no significant difference compared to control. Histopathological, slightly hydrophilic degenerative was observed in all organs. In conclusion, Pulp Out showed low acute toxicity in Wistar rats.

1. Introduction

Utilization of herbs or herbal medicine has lately increased, and according to World Health Organization (WHO), about 80 % of world population, in particular developing countries in Africa or Asia depends on natural herbs for health care due to economical, easy to get and processed, and relatively minimal adverse reactions [1,2]. Currently, herbal medicine has also been adopted and practiced as complementary and alternative medicine (CAMs) in Europe, America, and Australia for healthy life [3].

In some countries, medicinal plants are used as an alternative for oral hygiene maintenance and antimicrobial agents [4]. Some herbs have also been studied to have the potency to be developed as a pulp devitalizing agent to devitalize dental nerves in root canal treatment. Our previous study observed that these combined herbal extracts, namely Pulp Out, caused pulpal cell death in animal study following its application in exposed pulp which showed similar image of cell death of commercial pulp devitalizing agent, marked by the presence of changes and lysis in the cell nucleus [5].

The natural extract blend Pulp Out, which includes sidaguri, melittin, and jatropha sap, is thought to hasten the devitalization of pulp cells. Necrosis develops after a few days of the administration, as opposed to chemical-based devitalization agents like paraformaldehyde. This could be attributed to paraformaldehyde’s sluggish depolymerization process, which enters the pulp, irritates it, and eventually results in necrosis. Inducing cell lysis with Pulp Out relies on the dose used. More cells will be attributed to paraformaldehyde’s sluggish depolymerization process, which enters the pulp, irritates it, and eventually results in necrosis.
enhance the expression of caspase-3 and IL-1β. The number of blood vessels and their width is linked to increased inflammation after cell lysis [6].

Herbal medicine is generally considered safe; however, a toxicity test of herbal-based pulp devitalizing agent should be performed to examine any adverse effects against systemic organs that might occur before its application in humans [7]. The devitalizing agent applied might leak from temporised restoration into the oral cavity and get into the stom-ach, then spread to other organs that can harm the body [8]. A toxicity test is a measure used to examine any adverse effects of a substance against systemic organs that might occur prior to its application in humans [9].

The present study is aimed to evaluate the acute toxicity of Pulp Out on Wistar rats by observing the autonomic clinical signs, body weight, water and food consumption, hematologic values, histopathologic study of intestine, spleen, liver, and kidney organs after 2 weeks administered with Pulp Out. Further, this research also reported the impact of Pulp Out on periodontal and periapical tissue.

2. Materials and methods

2.1. Sample collection and preparation

The Pulp Out paste was prepared according to the previous methods described by Tanumihardja et al. [5]. The root of sidaguri and sap of jatropha were collected from Bone, South Sulawesi, Indonesia, in May 2021. The collected roots were washed well with running water, cut into small pieces, shade-dried, and powdered using a grinder. The dried roots were extracted using reflux method with ethanol 96 %. A rotary evaporator was used to evaporate and dry the extracts, then kept in a vacuum desiccator till use. In comparison, melittin was purchased orator was used to evaporate and dry the extracts, then kept in a vacuum desiccator till use. In comparison, melittin was purchased.

2.2. Animals use and care

Male healthy Wistar rats of 135–150 g body weight and age 12 weeks, bred locally in the animal holdings of the Department of Pharmacology, Sekolah Tinggi Ilmu Farmasi Makassar, were used in experiment. The animals were housed in standard plastic cages for at least 7 days prior to the start of the study to allow for acclimatization under natural atmospheric conditions. The animals were also fed with standard laboratory (BR-1 (Broiler-1) diet was the product of PT Japfa Comfeed Indonesia Tbk. BR-1 diet consists of 21.5 % protein; ≤ 12% moisture content; ≥ 5 % fat; ≤ 5 % crude fiber; ≤ 7 % ash; 0.8–1.1 % calcium; ≥ 0.5 % phosphorus, and 2950–3050 kcal/kg metabolic energy.) and water ad libitum, maintained under controlled temperature (23 °C ± 4 °C), 50–55 % relative humidity, and a 12 h light:12 h dark cycle. They were also given two weeks to adapt before the tests began. The procedure for animal care was adopted by the Ethics Commission of the Dental and Oral Hospital, Faculty of Dentistry, Hasanuddin University (No. 0112/PL.09/KEPK FKG-RSGM/UNHAS/2020).

2.3. Dosing of animals

1. Dosage of 50 mg/kg BW contain sidaguri 24 mg/kg BW, jatropha 24 mg/kg BW, and melittin 2 mg/kg BW;
2. Dosage of 500 mg/kg BW contain sidaguri 240 mg/kg BW, jatropha 240 mg/kg BW, and melittin 20 mg/kg BW; and
3. Dosage of 2500 mg/kg BW contain sidaguri 1200 mg/kg BW, jatropha 1200 mg/kg BW, and melittin 100 mg/kg BW.

2.4. Pulp Out application on periodontal and periapical tissue

For the experiment, 12 animals were randomly divided into two specifics: untreated group (control) and experimental groups receiving Pulp Out. Briefly, the animals were inserted once Pulp Out on periapical tissue. After 3 days, an overdose of ketamine and xylazine (300:30 mg/kg BW; i.p.) was used to euthanize the animals. The periodontal and periapical tissue were removed, and histopathological analyses were performed.

2.5. Median lethal dose (LD₅₀) determination and sighting study

The median lethal dosage (LD₅₀) was determined using Organization for Economic, Co-operation and Development (OECD) Test Guideline (TG) 425 [10], with an earlier reported LD₅₀ value as a starting point [11]. Due to the absence of mortality at 2500 mg/kg BW, a confirmatory test was done to corroborate the observation. The sighting investigation used the LD₅₀ value as a guide and a functional observational battery (FOB) to determine the optimal doses for acute and repeated dose toxicity testing and humane endpoint criteria.

2.6. Single dose (acute) toxicity study

Twelve male rats were used in this study, randomly allocated into 4 groups (n = 3) and the study procedure was adapted from OECD TG 423 [12]. Using an oral gavage, the graded doses of the Pulp Out were 50, 500, and 2500 mg/kg BW and distilled water (control) were orally administered to rats following an overnight fast. The volume of administering doses was not more than 1.0 mL/100 g BW. Cage side observation using FOB was monitored continuously for the first 30 min, then at regular 30 min intervals for the next 4 h, then regularly thereafter till 24 h and daily till day 14. The body weight of each animal was determined baseline, on day 7 and 14 thereafter. On day 14, rats were sacrificed for sample collection.

2.7. Blood collection

Rats were sacrificed by cervical dislocation. The jugular vein was exposed and cut with a sterile scalp knife and the blood were bled into either a 1.5 mL of EDTA-K3-coated and without anticoagulant specimen bottles. In contrast, samples of organs (spleen, intestine, liver, and kidney) were collected and preserved in 10 %v/v formalin in phosphate buffer saline (PBS) pH 7.4 for histopathological assessment.

2.8. Haematological assays

Haematological analysis of the blood samples was performed using an automated haematology analyzer (2800 Hematology Auto-Analyzer). Parameters which were evaluated included white Blood Cell (WBC), red blood cell (RBC), hemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet.

2.9. Biochemical assays

Blood plasma biochemical assays were obtained from the blood samples following centrifugation at 3000 rpm for 5 min. The blood level of serum glutamic oxaloacetic transaminase or SGOT by GOT (ASAT) IFCC mod. liquiUV, serum glutamic pyruvic transaminase or SGPT by GPT (ALAT) IFCC mod. liquiUV, urea by Urea liquiUV, and creatinine by Creatinine (enzyme) liquicolor were assessed by a Humalyzer 4000.

2.10. Histopathological examination

All the organ specimens from each rat were immediately stored in 10 %v/v formalin in PBS and dehydrated using increasing concentrations of
isopropyl alcohol (70–100 %). Paraffin sections at 5 µm thickness were made from the paraffin-embedded organs using a Leica rotary microscope (Bright BS143 Huntington, England). This was followed by routine staining with hematoxylin and eosin (HE), which involved the process of deparaffinization, hydration, staining, rinsing, and clearing in xylene. Slides were viewed under a light microscope with photomicrographs taken with a Leica DM750 Camera Microscope.

2.11. Data presentation and statistical analysis

Data were expressed as mean ± standard deviation (SD), and significant differences were determined using one-way analysis of variance (ANOVA) followed by the Tukey post hoc test using SPSS 19. Differences were considered significant at p < 0.05.

3. Result

3.1. Effects of Pulp Out on periodontal and periapical tissue

According to the content of Fig. 1, there was a decrease in the number of blood capillaries in Pulp Out group, but not significant with controls. One hallmark of acute inflammation is that initially the number of lymphocytes cells, osteoblasts, and osteoclasts in Pulp Out group were slightly increased compared to control. However, the statistics showed no significant effect on lymphocytes cells and both tooth cells.

3.2. Clinical signs recorded during the experiment

Initially, Pulp Out did not significantly affect the observed autonomic clinical signs at 50 and 500 mg/kg BW. However, when the doses of 2500 mg/kg BW were administered, autonomic clinical signs were observed on days 13 and 14 of diarrhea and piloerection, respectively (Table 1). The animals exhibited diarrhea and piloerection without any signs like catalepsy, ataxia, tremor, lacrimation, salivation, and diuresis during the test.

3.4. Effects of Pulp Out on biochemical indices

The effects of single-dose administration of Pulp Out on biochemical parameters are depicted in Fig. 3. Generally, administration of Pulp Out at 50, 500, and 2500 mg/kg BW in rats induced a slight increase in all parameters such as hepatic biomarkers: SGOT (Fig. 3A), SGPT (Fig. 3B), and kidney function markers: urea (Fig. 3C) and creatinine (Fig. 3D). However, the parameters scraped but still reached a normal level and were insignificant compared to the control.

3.5. Effect of the Pulp Out on haematological parameter indices

In this acute toxicity study, the haematological parameters, such as WBC, RBC, Hb, HCT, MCV, MCH, MCHC and platelet of the treated groups (50, 500, and 2500 mg/kg BW) were within the reference range for rats. The values in the Pulp Out-treated rats were not significantly different from the control (Table 2).

3.6. Effects of the Pulp Out on the spleen

For congestion category, photomicrograph of the spleen showed focal type in 1 rat at dose 50 and 500 mg/kg BW, and 2 rats at 2500 mg/kg BW, while the multifocal type was observed in 1 rat of 500 mg/kg BW. There was no diffuse type in all treated groups (Table 3 and Fig. 4).

3.7. Effects of the Pulp Out on intestine

For inflammation category, photomicrograph of intestine showed focal type in 1 rat at dose 50 and 500 mg/kg BW, while the diffuse type was observed in 1 rat at dose 50 and 2500 mg/kg BW. There was no fat degeneration category in a photomicrograph of the intestine for all treated groups, while a multifocal type of necrosis category was observed in 2 rats at a dose of 500 mg/kg BW (Table 4 and Fig. 4).

3.8. Effects of the Pulp Out on liver

Hydrophilic degeneration involved 25 % of liver disorder in 2 rats at a dose 50 mg/kg BW, 25–50 % in 1 rat of all treated groups, 51–75 % in 1 rat at a dose 500 mg/kg BW, and > 76 % in 1 rat at dose 500 and 2500 mg/kg BW while no hydrophilic degeneration was observed in 1 rat at 2500 mg/kg BW and control group. Hydrophilic degeneration

Table 1: Effect of Pulp Out on observed autonomic clinical signs.

| Parameters            | Control | Dose (mg/kg BW) |
|-----------------------|---------|-----------------|
|                       |         | 50              | 500     | 2500   |
| Ataxia                | –       | –               | –       | –      |
| Diarrhoea             | –       | –               | –       | –      |
| Diuresis              | –       | –               | –       | –      |
| Tremor                | –       | –               | –       | –      |
| Catalepsy             | –       | –               | –       | –      |
| Lacrimation           | –       | –               | –       | –      |
| Salivation            | –       | –               | –       | –      |
| Piloerection          | –       | –               | +       | –      |

Note: (–) not detected; (+) observed.

Fig. 1: Effect of Pulp Out on the number of capillaries, lymphocytes cells, osteoblasts, and osteoclasts. Slight lower capillaries were observed in Pulp Out-treated group, while the number of lymphocytes cells, osteoblast, and osteoclasts were increased. Photomicrograph of sections of periapical tissue was 100x magnification, while black arrow in each photomicrograph described the related cells.
involved 25% of kidney disorder in all rats at dose 50 mg/kg BW, 1 rat at 500 mg/kg BW and 2 rats at dose 2500 mg/kg BW; involved 25–50% in 1 rat at 500 and 2500 mg/kg BW, while no hydrophilic degeneration was observed in 1 rat at dose 500 mg/kg BW and control group (Table 5 and Fig. 4).

3.9. Effects of the Pulp Out on kidney

Hydrophilic degeneration involved 25% of kidney disorders in all rats of group 1, 1 rat of group 2 and 2 rats in group 3; involved 25–50% in 1 rat of group 2 and group 3, while no hydrophilic degeneration was observed in 1 rat of group 2 and control group (Table 5 and Fig. 4).

4. Discussion

Traditional medicine has long been practised in primary health care. However, the herbs are foreign substances in the body that need to be screened for their toxicity and to get scientific validation for its side effects produced by the herbs [13]. The pre-clinical toxicity testing helps before initiating the clinical evaluation of investigational products [14]. For clinical use, Pulp Out will be applied in the prepared tooth cavity and covered with temporary restoration for 1–2 weeks. During this period, Pulp Out may leak, penetrate into periapical tissue, and cause inflammation as a biological response of the immune system. However, lymphocytes cells and the number of capillaries showed no significant differences compared to control group. Number of osteoblasts and osteoclast in periapical tissue were also unaffected. This can be assumed that inflammation has already subsided and proceeded to proliferative phase.

The toxic effects of Pulp Out observed visually on the basis of body weight, water, and food intake showed no significant differences from the control group. This can be assumed that the metabolism of water and food in all treated groups was not affected by the presence of Pulp Out, which could also be linked to no weight loss observed in all treated groups. Response of observed autonomic clinical signs only showed a slight effect, while hematologic evaluation showed some variation in increased and decreased red blood cell, white blood cell, and haemoglobin values but consistently increased thrombocyte value without any significant differences compared to control. Extract of root of sidaguri has been practiced in relieving toothache by drinking the boiled water of the extracts, while jatropha sap and melittin are applied directly to the ached teeth. These suggested the extracts do not harm the body [15].

Intestine and spleen are organs exposed when foreign substances/xenobiotics enter the digestive system. The intestine plays a role in immune reactions to foreign substances and has an epithelium which is the first layer of defense against pathogens in the intestinal mucous, while spleen is responsible in initiating the immune system [16]. In this study, intestinal inflammation was observed in some of treated animals after Pulp Out application. When xenobiotic/foreign substances enter the intestine, they will undergo enterohepatic circulation resulting in repeated injury to the intestine that might cause cell damage. The damaged cells should be replaced in order to restore homeostasis after an acute or chronic attack. Multifocal necrosis observed at 500 mg/kg BW might be assumed that intestinal mucosa continuously undergoes
cell turnover without causing toxicity. This was confirmed with no fat degeneration in intestine found in histology study.

The spleen is one of the target organs for oral xenobiotics, and congestion is an early indication of reversible spleen inflammation that can return to normal when is no longer exposed to xenobiotic [17]. Splenic congestion is common without any apparent cause and is seen as an excessive accumulation of erythrocytes in the red pulp sinus. There are many causes of splenic congestion in Wistar rats including bacterial infection, cardiovascular disease, mononuclear cell leukemia, erythrocyte damage, euthanasia methods and necropsy procedures [18,19]. In this study, focal and multifocal congestion of the spleen was observed after the administration of various doses of pulp-out. This may indicate that spleen works in response to exposure of oral xenobiotics by filtering the blood and removing damaged red blood cells [20]. When this is linked to erythrocyte value, 50 and 500 mg/kg BW showed decreased of erythrocyte however the value increased in 2500 mg/kg BW. It can be assumed that pulp out is not toxic to spleen.

Liver is an essential organ for detoxifying chemicals and metabolizing drugs that renders it susceptible to the toxicity of these substances (Friedman et al., 2003). Impairment of liver function is marked as a rise in enzyme tests either SGOT or SGPT. SGOT level in this study showed elevation in a dose dependent manner without significant difference compared to control. According to Washington and Van Hoosier [21], the increase of the SGOT level might be caused by several factors as xenobiotic induction, liver injury, heart, kidney, or muscles. Similar to SGOT, SGPT level also showed a slight increase except rats administered with 500 mg Pulp Out, however no statistically significant difference compared to control group. This could be an individual response to the extracts. SGOT and SGPT were released into blood circulation due to necrosis or damage of membrane cell. Histologically, observation of liver tissue showed hydrophilic degeneration in all treated groups with various degrees. Hydrophilic degeneration is a common feature in acute toxicity that will disappear when xenobiotic exposure was stopped and the impaired liver cells return to normal [22]. Hepatotoxicity is determined when a significant increase of SGOT and SGPT accompanied with liver cell necrosis. Johns [23] suggested that histologic evaluation provided better prognostic information than blood tests. It could be assumed that pulp out has limited side effects to the liver.

The kidney is another important organ with the main function to excrete the residual metabolic product into urine [24]. Kidney failure
The administration of Pulp Out. The function of kidney system. Protein need of a rat is about 12% [25], and in this study the protein intake was 17% that might explain the increased of urea concentration. Similar to urea, creatinine concentration is also affected by muscle mass. Therefore, the ratio between urea and creatinine, known as BCR ratio, the blood urea nitrogen to creatinine ratio is usually used to distinguish kidney diseases from acute kidney injury. BCR ratio > 20 mg/dL was observed in 2500 mg/kg BW. Many causes can be related to the increase of urea, including decreased of blood flow to the kidney, dehydration, heart failure, gastrointestinal bleeding, catabolic status due to trauma, severe infection, starvation, and corticosteroid [26,27].

Urea and creatinine concentration would be significant when alteration of kidney histology [28]. The result in this study showed hydrophilic degeneration under histologic observation of kidney organ and occupied mostly 25%. Hydrophilic degeneration is an initial reversible injury on tissue that occurs when cell is not capable to maintain ionic and fluid homeostasis. It was suggested that application of Pulp Out had a relatively mild effect to kidney organ. This supported the studies of Konaté et al. [29] and Ramalho et al. [30] that Sida rhombifolia had a limited, rapid, and reversible toxicity, while Abdelgadir and Staden [31], Siregar (2015), and Okechukwu et al. [32] found that Jatropha curcas L sap is cytotoxic in moderate category without causing nephrotoxicity in Wistar rats.

Similar to microorganisms, injured/dead cells caused by toxic agents, stimulate inflammation through a number of different mechanisms. Cells contain multiple danger signals that are released upon death and disintegration of the plasma membrane. These are recognized as debris associated molecular patterns (DAMPs) by TLRs as cellular receptors that stimulate the generation of proinflammatory mediators such as IL-1, TNF-α, IL-6, while other molecules released stimulate the generation of mediators from extracellular sources [33,34].

The resulting mediators orchestrate the inflammatory response, eliciting in various vascular and cellular components. Dilatation of blood vessels and extravasation promote the recruitment of leucocytes. Fluid leakage helps to dilute, neutralize, and drain away soluble injurious agents. On the other hand, mediators released by inflammatory cells also promote repair and restore the tissue integrity for the growth of many of these cells. The fibrinogen that leak from inflamed vessels is converted into fibrin that provide scaffold for the growth of these cells. Dying cells may also provide signals that help mobilize adaptive immunity to antigens present in the affected tissue. Dead cells could activate dendritic cells and promote the generation of CD4 and CD8 T-cell response to immunogenic antigens that are present in or around the dying cells [33,35].

5. Conclusion

Under the limitations of this study, our results have demonstrated that application of 50 mg and 500 mg/kg BW of Pulp Out are relatively safe, without any adverse effect to periapical tissue. In addition, observed autonomic clinical signs, body weight, food consumption, water intake, hematological parameter indices, biological indices are also not affected, while organs examined histologically including spleen, intestine, liver and kidney are only slightly affected which are reversible in nature. Pulp Out can be further developed as tooth-pulp devitalizing agent.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

### Table 2

Effect of Pulp Out on haematological parameters.

| Haematology parameter | Dose (mg/kg BW) | Control |
|-----------------------|----------------|---------|
|                       | 50            | 500     | 2500    |
| White Blood Cell (WBC) | 20.53         | 14.67   | 16.20   | 17.17   |
|                       | ± 8.99        | ± 4.42  | ± 3.46  | ± 5.71  |
| Red Blood Cell (RBC)  | 8.40          | 8.00    | 8.77    | 8.46    |
|                       | ± 0.55        | ± 0.06  | ± 0.42  | ± 0.60  |
| Haemoglobin (Hb)      | 14.80         | 13.93   | 15.30   | 14.97   |
|                       | ± 0.60        | ± 0.40  | ± 0.10  | ± 1.21  |
| Hematocrit (HCT)      | 49.60         | 46.37   | 50.30   | 48.77   |
|                       | ± 3.31        | ± 0.42  | ± 0.56  | ± 4.44  |
| Mean Corpuscular Volume (MCV) | 59.13 | 57.90 | 57.43 | 57.60 |
|                       | ± 4.72        | ± 0.70  | ± 2.50  | ± 2.52  |
| Mean Corpuscular Hemoglobin (MCH) | 17.67 | 17.40 | 17.47 | 17.70 |
|                       | ± 1.25        | ± 0.62  | ± 0.87  | ± 0.62  |
| Mean corpuscular haemoglobin concentration (MCHC) | 29.87 | 30.07 | 30.43 | 30.70 |
|                       | ± 0.90        | ± 1.10  | ± 0.55  | ± 0.36  |
| Platelet              | 726.67        | 747.00  | 738.00  | 546.33  |
|                       | ± 177.72      | ± 132.53| ± 161.89| ± 257.90|

### Table 3

Semiquantitative scoring of spleen histopathologic changes in congestion after administration of Pulp Out.

| Dose (mg/kg BW) | Normal | Focal | Multifocal | Diffuse |
|----------------|--------|-------|------------|---------|
| 50             | 2      | 1     | –          | –       |
| 500            | 1      | 1     | 1          | –       |
| 2500           | 1      | 2     | –          | –       |
| Control        | 3      | –     | –          | –       |

Fig. 4. Photomicrograph of sections of spleen (100× magnification), intestine, liver, and kidney (400× magnification) in control and treated groups. Congestion in spleen, inflammation in intestine, vascular/hydrophilic degeneration in liver and vascular/hydrophilic degeneration in kidney as marked by black arrows.
Table 4
Semiquantitative scoring of intestine histopathologic changes after administration of Pulp Out.

| Dose (mg/kg BW) | Inflammation | Fat degeneration | Necrosis |
|----------------|--------------|------------------|----------|
|                | Normal | Focal | Multifocal | Diffuse | Normal | Focal | Multifocal | Diffuse | Normal | Focal | Multifocal | Diffuse |
| 50             | 2      | –     | –         | 1       | –      | 3     | –         | –       | –      | 3     | –         | –       |
| 500            | 2      | 1     | 1         | 3       | 1      | 1     | 1         | –       | –      | 1     | 1         | –       |
| 2500           | 1      | 1     | 1         | 3       | –      | 3     | –         | –       | –      | 3     | –         | –       |
| Control        | 3      | –     | –         | 3       | –      | 3     | –         | –       | –      | 3     | –         | –       |

Table 5
Semiquantitative scoring of kidney and liver histopathologic changes in hydrophilic degeneration after administration of Pulp Out.

| Dose (mg/kg BW) | Hydrophilic degeneration | Kidney |
|----------------|--------------------------|--------|
|                | Normal | Focal | Multifocal | Diffuse | Normal | Focal | Multifocal | Diffuse | Normal | Focal | Multifocal | Diffuse |
| 50             | 0      | 25%   | 50%       | 75%     | > 75%  | 0     | 25%       | 50%     | 75%     | > 75%  |
| 500            | 1      | 1     | 1         | 1       | 1      | 1     | 1         | 1       | 1      | 1     | 1         | 1 |
| 2500           | 3      | –     | –         | –       | –      | –     | –         | –       | –      | –     | –         | – |
| Control        | 3      | –     | –         | –       | –      | –     | –         | –       | –      | –     | –         | – |

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Data Availability

Data will be made available on request.

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