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# Repeated dose 28-day oral toxicity study of a botanical composition composed of *Morus alba* and *Acacia catechu* in rats

Mesfin Yimam[a,*], Ping Jiao[a], Mei Hong[a], Lidia Brownell[a], Hyun-Jin Kim[b], Young-Chul Lee[b], Qi Jia[a]

[a] Unigen Inc., 3005 1st Ave., Seattle, WA 98121, USA
[b] Unigen Inc., #450-86, Maebong-Ro, Dongnam-Gu, Cheonan-Si, Chungnam 330-863, South Korea

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ABSTRACT

Patients with osteoarthritis experience debilitating pain and loss of joint function that requires chronic treatment. While nonsteroidal anti-inflammatory drugs (NSAIDs) have been effective for temporary symptomatic relief, their long term usage has been limited by their associated side-effects. UP1306, a standardized novel composition from the extracts of root barks of *Morus alba* and the heartwoods of *Acacia catechu*, has been used in over the counter joint care dietary supplements as a safer alternative. These two medicinal plants have long track records of safe human consumption. Here we evaluated the potential adverse effects of orally administered UP1306 in Sprague Dawley rats following a 28-day repeated oral dose toxicity study. UP1306 at doses of 500, 1000 and 2000 mg/kg/day were administered orally to rats for 4 weeks. A 2-week recovery group from the high dose (2000 mg/kg) and vehicle treated groups were included. No morbidity or mortality was observed for the duration of the study. No significant differences between groups in body weights, food consumption, hematology, clinical chemistry, organ weights, gross pathology and histopathology were documented. Minor aberrations from the normal observed for the main groups were considered reversible as they were not evident in the recovery period. In conclusion, the no-observed-adverse-effect-level (NOAEL) of UP1306 was considered to be the highest dose tested, 2000 mg/kg/day, both for male and female rats.

## 1. Introductions

*Morus alba (M. alba)* and *Acacia catechu (A. catechu)* have been used separately in traditional medicines and contemporary pharmaceutical compounding for multiple indications.

*Morus alba L* (Family: Moraceae), the mulberry or white berry plant, is native to northern China, and has been cultivated and naturalized elsewhere, from India through middle east to Southern Europe, and recently to North America. The root-bark of *Morus alba* that is used in traditional medicine is known as Sang Bai Pi or Cortex Mori (Pharmacopeia of the People's Republic of China, 2005). This herb is also known as Pong-na-moo in Korean and Sohakuhi in Japan. In contemporary pharmacological research, *Morus alba* root-bark has been reported to have antibacterial (Gunjal et al., 2015), antioxidant and hypoglycemic (Raman et al., 2016; Wang et al., 2013), hypolipidemic, neuroprotective, antiulcer, analgesic (Jo et al., 2014; El-Beshbishy et al., 2006; Eo et al., 2014) and anti-inflammatory activities (Chan et al., 2016). Some of the prenylated flavonoids and stilbenoids such as morusin and mulberroside A are unique to *Morus* plants (Yang et al., 2014).

*Acacia catechu* (L.f.) Willd (also known as “black catechu” or “katha) is from the Leguminoseae family native to India and Burma and also found in China and parts of Bangladesh. The heartwood hot water extract of *Acacia catechu*, is mainly used in India as an ingredient of chewing gums (Singh and Lal, 2006). Traditionally, *Acacia catechu* has been a component in Ayurveda medicine for the purpose of relieving toothache, sore throat, gum ulceration, digestion aid, and hepatoprotection (Nadkarni, 1976; Jarald et al., 2009), as well as healing bleeding gums and canker sores. In the Chinese Pharmacopeia, the dry extract of stems and woods of *Acacia catechu* is used for healing ulcers, weeping skin disease, diarrhea, traumatic injuries, and hemorrhoids. Thai medicine also lists *Acacia catechu* for its anti-diarrheic properties (Sawangjaroen and Sawangjaroen, 2005). Catechin is a major flavan in *Acacia* plants (Abdulrazak et al., 2000).

Multiple toxicological studies of *M. alba* and *A. catechu* extracts have been documented separately and found to be relatively non-toxic. For instance, acute toxicity study conducted in mouse, LD50 (lethal dose) of catechin was found to be 2170 mg/kg after oral administration

* Corresponding author.
*E-mail address:* myimam@unigen.net (M. Yimam).

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(KSRNAM Kiso to Rinsho, 1987). Other studies had also established LD50 of catechin as greater than 10 g/kg for mouse and rat when administered orally, 68 mg/kg for mouse or 1084 mg/kg for rats when given intraperitoneal, 5 g/kg for mouse or rats when applied topically, and 100 mg/kg for mouse or rat when infused intravenously (Yakuri, 1982). Supplementary short term toxicity data also showed that the dietary administration of epigallocatechin gallate (EGCG), similar class as catechin, to rats for 13 weeks was not toxic at doses up to 500 mg/kg/day (Isbrucker et al., 2006a). To evaluate effect on reproductive toxicity, catechin was administered to mated female rats from gestation days 6–17 at oral doses of 200, 600, and 2000 mg/kg/day. In this study, catechin did not impact the mean gravid uterine weights or intrauterine growth, survival, fetal malformations or developmental variations (Morita et al., 2009). In another study, when female C57BL/6J mice were provided with 2% (+)-catechin in diet prior to, during pregnancy and lactation, the authors reported that offspring food intake, body weight, litter size, survival, sex, and skeletal development were unaffected even though some changes in liver mineral concentration for copper and iron were noticed (Lesser et al., 2015). Isbrucker et al. have also reported that when Wistar rat dams were fed a standard powdered diet supplemented with epigallo-catechin-gallate (EGCG) daily between gestation days 6–20 at 0, 100, 300, and 1000 mg/kg/day showed no difference in fetal weight or incidence of external, visceral, or skeletal malformations (Isbrucker et al., 2006b). In fact, some studies have suggested the antioxidant potential of catechins during pregnancy to protect the fetus from oxidative stress (Chan et al., 2016).

Similarly, previously, *M. alba* root bark extract was tested in a 28-day repeated dose oral toxicity study. In the study male and female rats were gavaged with *M. alba* extract at 1,000, 2000 and 4000 mg/kg/day for 4 weeks. No treatment-related mortality or adverse effects (per clinical observations, body weight/weight gain, food consumption, ophthalmoscopy, clinical pathology, gross pathology, organ weights, or histopathology) were observed, and no organs targets for adverse effects were identified. The highest dose tested (4000 mg/kg/day) was determined as the no observed adverse effect level for the study (Marx et al., 2016). In association, administration of *M. alba* root bark extracts at oral (p.o.) and intraperitoneal (I.P.) doses of 2, 5 and 10 g/kg. The same group also tested the effect of the extract administered intravenous (i.v.) at 2 and 5 g/kg. Both studies resulted in no mortality leading the authors to conclude the lethal doses of the fractions to be more than 5 (i.v.), 10 (i.p.) and 10 g/kg (p.o.) (Yamatake et al., 1976). In our lab, we tested the maximum tolerable dose at 5 g/kg in Sprague Dawley (SD) rats (N = 5 males and 5 females) and repeated dose14-day oral toxicity study (N = 10 males and 10 females SD rats) at 500, 1000 and 2000 mg/kg/day of extracts from *M. alba or A. catechu.* We monitored changes in physical appearance, food consumptions, body weight, clinical chemistry, hematology, absolute and relative organ weight. In both studies, no sign suggestive of toxicity was observed.

The present study was designed to evaluate the safety profile of UP1306, a standardized bio-flavonoid composition of *M. alba* and *A. catechu* extracts, after a 28-day repeated dose oral administration to SD rats.

## 2. Materials and method

### 2.1. The composition

The original dried root bark of *Morus alba* L. var. *multicaulis* (Perr.) Loudon in the Moraceae family was collected from Chongqing, China and identified by professor Shou-Yuen Zhao from Si-Chuan Chinese Traditional Medicine Research Institutes. A voucher of specimen of *Morus alba* (P00329) was deposited at the plant library of Unigen Inc. Seattle, WA, USA. The recollected *Morus alba* root barks were characterized and confirmed in comparison with the original voucher specimens.

The dried branch and trunk of *Acacia catechu* Willd. A. Suma (Senegalia catechu (L.f.) P.J.H Hunter & Mabb) in the Leguminosae family was initially collected from Yunnan, China and identified by professor Shou-Yuen Zhao from Si-Chuan Chinese Traditional Medicine Research Institutes. A voucher of specimen of *Acacia catechu* (P00530) was deposited at the plant library of Unigen Inc, Seattle, WA, USA. The recollected heartwoods of *Acacia catechu* from Distt Kangra Himachal Pradesh, India were characterized and confirmed in comparison with the original specimens.

Detailed procedure for the preparation of the composition has been described in the United States patent #20150072953 (Brownell et al., 2015). Dried *Morus alba* root barks were cut, crushed, and then extracted with approximately seven-fold volume of 70% ethyl alcohol in water at 100 °C for 4 h three times to give *Morus alba* 70% EtOH extract powder with a yield of 19.6% (w/w). The standardized morus extract contains no less than 4% Mulberroside A and no less than 3% of total bioflavonoids including Kuwanon G, Albanin G and Morusin. Catechins enriched *Acacia* extract was obtained by repeated crystallization from the aqueous extract of *Acacia catechu* heartwoods with a yield of 14.5% (w/w). (+)-Catechin was identified as the major active flavan in the *A. catechu* extract. The Acacia extract was standardized as no less than 65% of catechin and a minor enantiomer epicatechin. Compositions UP1306 was prepared by mixing the standardized extracts of *Acacia catechu* heartwoods (no less than 65% catechins) and *Morus alba* root barks (no less than 7% stilbenes and bioflavonoids) at a ratio of 1:2 by weight with no less than 15% catechins and 2% stilbenes and bioflavonoids. The 1:2 ratio is the ratio at which the maximum efficacy in anti-inflammatory, anti-pain sensitivity and cartilage protection activities observed.

### 2.2. Animals

Male and female Sprague–Dawley rats at 7 weeks of age were purchased from OrientBio Co., Ltd. (South Korea) and acclimated to the laboratory conditions for a week. Following quarantine and acclimation, 50 males and 50 females were selected and assigned to 4 main groups (10 animals/sex/group for Groups 1, 2, 3 and 4) and 2 recovery groups (5 animals/sex/group for Groups 1 and 4). The recovery groups were assigned to control and high dose (2000 mg/kg) for both genders. Animals were distributed in an attempt to equalize mean group body weights. Rats weighed 220–240 g (males) and 150–175 g (females) at test initiation. Rats (2/cage) were housed in a Polycarbonate cage, 260W × 420D × 180H (mm) in specific pathogen free (SPF) Room #2 and individually identified by numbers on their tails. Each cage was covered with wire bar lid and filtered top (Allentown, NJ, USA). Individual cage was identified with a cage card indicating project number, test article, dose level, group, and an animal number. The Harlan T7087 soft cob beddings were used and changed at least twice weekly. Animals were provided with fresh water and rodent chow diet # 2917c (Harlan Teklad, USA) *ad libitum* and were housed in a temperature-controlled room (23.1 °C) with humidity 50 ± 10%, and 10–15 filtered air changes per hour on a 12 h light-dark cycle. The present study was conducted based on -" Organization for Economic Co-operation and Development (OECD) GUIDELINE FOR THE TESTING OF CHEMICALS (2008): Repeated Dose 28-day Oral Toxicity Study in Rodents, Guideline 407" and -"KFDA (Korea Food and Drug Administration) (1998): Guidelines for toxicity studies of drugs, Notification No. 1998–116, Seoul, Korea". This study was aslo reviewed and approved by the Institutional Animal Care and Use Committees (IACUC) of Unigen, Inc. based on Animal Protection Act (Approval No. UIK 11303).

### 2.3. Groups and dosing

The rats received UP1306 at oral doses of 0 (control), 500, 1000 and 2000 mg/kg/day. The test article was mixed in a solution of 0.5% carboxymethylcellulose (CMC) in distilled water. The test article was