Safety Evaluation of a Combination, Defined Extract of *Scutellaria baicalensis* and *Acacia catechu*

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ABSTRACT

A combined extract containing primarily baicalin from *Scutellaria baicalensis* and (+)-catechin from *Acacia catechu* used in both joint supplements and a prescription medical food was tested for safety. Cytotoxicity testing in THP-1 monocytes showed limited cell death compared to NSAIDs. Acute and subchronic toxicity testing demonstrated no abnormalities in any toxicological endpoints examined including animal body weights, gross organ pathology and tissue histology, and blood chemistries or serology. The extract, when dosed in Fischer 344 rats, a model for gastric toxicity of NSAIDs, showed no evidence of ulceration. No mutagenicity or drug interactions were seen by AMES and CYP450 enzyme inhibition, respectively. When the extract was compared with placebo after administration to a healthy human population, no changes in blood chemistry or serology were observed. Based on these findings, the combined extract with baicalin and catechin appears to possess a safety profile that justifies further testing in humans.

PRACTICAL APPLICATIONS

A specific ratio of *S. baicalensis* and *A. catechu* metabolically balances the inhibition of COX-1 and COX-2 enzymes and provides a comparable amount of 5-lipoxygenase (5-LOX) enzyme inhibition in the conversion of arachidonic acid (AA) to inflammatory metabolites (unpublished). This mechanism of action may yield fewer side effects than traditional non-steroidal anti-inflammatory drugs and selective COX-2 inhibitors produced by their generally specific inhibition of either COX enzyme (Grosser et al., 2006). Simultaneous inhibition of 5-LOX may also avoid a “shunt” of AA metabolism down the LOX pathway thus reducing side effects related to excess, unopposed production of inflammatory vasoconstrictive as well as

chemoattractive leukotrienes. The net result should be an effective anti-inflammatory metabolic product for the management of osteoarthritis with an improved toxicity profile and lower requirement for use with gastroprotective therapies.

Key Words: flavonoid, baicalin, catechin, osteoarthritis, cyclooxygenase, lipoxygenase, dual inhibition, medical food.

INTRODUCTION

Flavonoids, ubiquitous polyphenolic compounds found in plants, have been part of the human diet throughout the course of evolution. More than 4000 distinct, natural flavonoids have been identified thus far and have been consumed as part of common foods, teas, wines, traditional medicines and pharmaceutical drugs (Middleton et al., 2000). Flavonoids contribute to flavor in foods and spices, pigments found in fruits and vegetables, antimicrobial and antiviral agents used to fight infection, antioxidants, and pharmaceutical agents for the reduction in chronic diseases such as heart disease, Type II diabetes and dementia (Cowan, 1999; Knekt et al., 2002; Lule and Xia, 2005). Among their many activities, flavonoids also have both anticarcinogenic and antimutagenic properties (Lule and Xia, 2005; Moon et al., 2006). Two flavonoids, baicalin from *Scutellaria baicalensis* (Chinese skullcap) and (+)-catechin from *Acacia catechu* (black catechu) have been used separately in many traditional medicines and pharmaceutical products**,** especially in Asia**,** for a variety of purposes including anti-inflammatory, antiviral, antibacterial, anticancer and cardiovascular applications (Razina et al., 1989; Mahmood et al., 1993; van Loon, 1997; Cowan, 1999; Lee et al., 2000; Shigeta, 2000; De Clerq, 2005; Huang et al., 2005).

S. baicalensis root**,** in various dosage forms**,** has shown very low toxicity when administered orally, but higher toxicity when administered intravenously. The subacute oral toxicity of dried aqueous decoctions of two traditional Japanese multi-herb formulas was studied in rats (Chinese skullcap plus other herbs). The results suggest maximum no-effect dose levels of >1 g/kg body weight/day (Kiwaki et al. 1989ab). Oral administration of the aqueous decoction of *S. baicalensis* alone in rabbits (10 g/kg body weight) or intravenous administration of an

ethanolic extract (2 g/kg body weight) caused sedation, but no deaths (Chang and But, 1987). Intravenous administration of an aqueous extract (2 g/kg body weight) initially produced sedative and hypnotic effects in rabbits. Death then occurred in all animals after 8–12 hours. When the dosage was reduced to 1 g/kg body weight, sedation occurred, but no death. A single dose of an aqueous extract (12 or 15 g/kg body weight; orally) in dogs caused no reactions during 48 hours of observation**,** with the exception of emesis in the higher dosage group (Chang and But, 1987). Oral administration of an aqueous extract (4 or 5 g/kg body weight, t.i.d.) in dogs for eight weeks did not cause any significant changes in routine blood tests and histology of internal organs. Loose bowel movements occurred in the higher dosage group, but symptoms disappeared following discontinuation of treatment (Chang and But, 1987). Intraperitoneal administration of the aqueous extract was mutagenic in mice at a dose of 4 mg/kg body weight (Yin et al., 1991). The median lethal dose in mice**,** following subcutaneous injection of an ethanolic extract**,** was 6 g/kg body weight, for isolated baicalin, 6 g/kg body weight. The LD_{50} of isolated baicalin in mice was 3.081 g/kg body weight following intraperitoneal injection (Chang and But, 1987).

For (+)-catechin from *A. catechu,* an LD_{50} for oral administration**,** was established as 2.17 g/kg of body weight in mice (Ksrnam, 1990). Similar studies reported by different companies in Japan give LD_{50} in rats by oral administration of >10 g/kg, by subcutaneous injection of >5 g/kg, by intraperitoneal injection of 1.084 g/kg and by intravenous injection >100 mg/kg with observations of dyspnea in some animals (Oyo Yakuri, 1982; NIIRDN Drugs, 1990). For a related catechin from green tea, epigallocatechin gallate (EGCG), oral doses of 2 g/kg were

lethal to rats whereas 200 mg/kg induced no toxicity (Isbrucker et al., 2006). Inclusion of EGCG in diets of rats over a 13 week period showed no toxicity at doses up to 500 mg/kg/day.

The objective of the current study is to establish a safety profile for the extracts containing a relatively pure combination of baicalin and (+)-catechin since they are being used as part of a specially formulated, prescription medical food for the dietary management of osteoarthritis, as well as in over the counter joint support supplements. Though extensive toxicological testing of *S. baicalensis* and *A. catechu* extracts**,** as well as the flavonoids, baicalin and (+)-catechin, from each plant have been performed separately and found to be relatively non-toxic (see above), they have never been investigated for their toxicity in combination, a requirement that medical professionals demand for a prescription product. We have, therefore, examined the *in vitro* toxicity of the combination extracts on cells, *in vivo* in rodent models, using the AMES assay for mutagenicity, CYP450 enzyme inhibition for potential drug interactions and finally in a pilot human clinical safety study to assess tolerance. These studies suggest that these compounds, in combination, are non-toxic and warrant further clinical investigation in humans.

MATERIAL AND METHODS

Preparation of extracts containing baicalin and catechin

Three different lots of *S. baicalensis* extracts from roots were used in toxicity experiments. The roots were extracted with 70% ethanol and then re-crystallized with an ethanol/water solvent. The *S. baicalensis* extract contained baicalin as the major component (82.2%) (Figure 1A), as well as other minor free-B-ring flavonoids: wogonin-7-O-G-glucuronide, oroxylin A -7-O-G-glucuronide, and baicalein. Baicalein, normally a digestive product of baicalin by intestinal flora**,** appears in small amounts in the root of *S. baicalensis*. Three different lots of catechin extract were obtained from repeated crystallization of an aqueous extraction of the heartwoods of an India medicinal plant, *A. catechu*, and used in toxicity experiments. (+)-catechin is the major component in the *A. catechu* extract at a content of 80.4% (Figure 1B)**,** plus a minor amount of its entantiomer, epicatechin, as well as other minor amounts of flavans.

Analysis of the extracts was performed separately by HPLC/PDA and LC/MS. The results showed a major compound, baicalin, from the *S. baicalensis* extract and (+)-catechin from the *A. catechu* extract by comparison with known standards. These compounds were then confirmed by ^{13}C NMR and ^{1}H NMR analysis respectively. The final UP446 formulation was a mixture of *S. baicalensis* and *A. catechu* standardized extracts at a ratio 4:1. The combined flavonoid content was analyzed by HPLC using a Phenomenex Luna 5μ C-18, 250mm x 4.6mm with a C-18 Security Guard cartridge in a column oven at 35^{o}C. The mobile phase had a flow rate of 1.0 ml/min and used an isocratic 1% phosphoric acid:acetonitrile ratio of 85%:15% for the first 7 min**,** and then a new gradient to 10%:90% from 7 min to 16.5 min**,** and then an isocratic 1%

phosphoric acid:acetonitrile gradient with a ration of 85%:15% for 7.5 minutes. The flavonoids were detected using a UV detector at 275 nm and identified based on retention time by comparison with known flavonoid standards.

Cell Toxicity

Cell toxicity was tested using a human THP-1 monocyte cell line (ATCC# TIB-202). Confluent monolayers were exposed to increasing concentrations of UP446, celecoxib, indomethacin, ibuprofen and aspirin in triplicate in concentrations between 0.2 to 100 μg/ml. Release of lactate dehydrogenase (LDH), constitutively produced in the cell, is proportional to the number of cells damaged, or that lyse due to cytotoxic effects of each compound (Schafer et al., 1997). Addition of a specific colorimetric substrate**,** and its conversion to a secondary metabolite, was measured by enzyme analysis (Oxford Biomedical Research; Korzeniewski and Callewaert, 1983). The percent cytotoxicity was then calculated and results plotted as % toxicity versus μg/ml of test material used in the assay.

Animals

Outbred ICR mice and Fischer 344 (F344; Harlan Inc.) male rats (young rats age 6 months and aged rats age 18 months) were used to determine toxic effects of UP446. Mice were received at 5 weeks of age and used after 1-week of acclimatization. Animals were housed 5 per cage and maintained under a light:dark schedule of 12h:12h. Rats were housed in groups of six per cage. Both were provided tap water and commercial rodent chow (Harlan Teklad 2019) *ad libitum*. Health status of the mice and rats was monitored once daily. Body weight, food, and water consumption were measured once per week. All animals were maintained under accepted

health standards in a facility approved by the Institutional Animal Care and Use Committee (IACUC) and were maintained in accordance with the G*uide for the Care and Use of Laboratory Animals* (NRC, 1996).

Test Article and Treatment

UP446 for the acute toxicity study in mice was prepared daily, vortexed into a fine suspension with deionized water, and repeatedly vortexed between gavages to maintain consistent dosing. UP446 for subchronic studies in mice and rats was formulated with NIH31 mouse/rat chow to achieve calculated doses of 50, 250 and 500 mg/kg/d body weight in mice and 34 mg/kg/d in rats based on average daily consumption of chow.

Acute Mice Toxicity

Randomly assigned groups of 10 male and 10 female ICR mice received 14 consecutive days of treatment with 2000 mg/kg/d of UP446 by gavage. Control mice received water alone.

Subchronic Mice Toxicity

Randomly assigned groups of 10 male and 10 female ICR mice received 13-wks of treatment with UP446 formulated diets at doses of 0, 50, 250 and 500 mg/kg/d. The test diet was confirmed to be stable and was fed *ad libitum*. The control mice received NIH31 mouse/rat chow with no added UP446. The dose was calculated based on average daily consumption of food.

Subchronic Aged Rat Toxicity

Randomly assigned groups of 12 young and 12 aged male F344 rats received doses of 0 and 34 mg/kg/d UP446 for 9-wks in formulated diets. The dose was calculated based on average daily consumption of food.

Observations and Examinations

Clinical and behavioral signs were observed once daily. After 2-wks of acute toxicity treatment in mice, 9-wks of subchronic toxicity treatment in rats, and 13-wks of subchronic toxicity treatment in mice, the animals were anesthetized with isoflorane, a gross necropsy was performed, blood samples were collected by cardiac puncture, and liver, kidney, duodenum and stomach were removed and stored in formalin and examined for histological changes by an independent pathologist.

Blood samples were analyzed for hematological parameters including: red blood cell count (RBC), white blood cell count (WBC), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and platelet count (PLT). Serum biochemical parameters analyzed were: total protein (TP), albumin (AlB), globulin (GlB), total bilirubin (T.Bil), total cholesterol (TC), glucose (gluc), blood urea nitrogen (BUN), creatinine (CRN), phosphorus (P), sodium (Na), chloride (Cl), potassium (K), calcium (Ca), alkaline phosphate (ALK) and alanine aminotransferase (ALT). At necropsy, all of the tissues and organs were observed macroscopically for abnormalities.

Cytochrome P450 Inhibition

UP446 was tested at a 10 μM concentration in a liver microsomal assay with endoplasmic reticulum (ER) fractions using spectrophotometric quantization of 7-benzyloxy-4-(trifluoromethyl)-coumarin as the substrate for CYP profiling (Crespi et al., 1997). The following isoenzymes are principally responsible for detoxification of drugs: CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4. Furafylline (for CYP1A2), sulfaphenazole (for CYP2C9), tranylcypromine (for CYP2C19), quinidine (for CYP2D6), and ketoconazole (for CYP3A4) were used as control compounds inhibiting each enzyme to 100% at a 10μM concentration in the CYP450 inhibition analysis (Moody et al., 1999).

AMES Test

The procedure described in the OECD guideline no. 471 was adapted and a modification of the AMES test was performed on UP446 (Maron and Ames, 1983; Gatehouse et al., 1994). Briefly, five histidine-dependent strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102) were used to evaluate the mutagenic potential of UP446 diluted in DMSO. The experiment was performed using the plate incorporation method, with one plate per dose level and three plates for each vehicle and untreated negative control. The experiment was performed at the dose levels of 5 to 5000 μg/plate (approximately half-log progression between the dose levels), both in the presence and absence of metabolic activation. The number of His^- to His^+ revertants was measured for each strain to determine the mutagenic potential for UP446, as well as the cytotoxicity elicited with increasing dose on all five histidine-requiring Salmonella strains.

Human clinical safety study

A pilot human clinical study of oral administration with UP446 was conducted at Georgetown University. This clinical study was a single-center, randomized, double-blind and placebo-controlled study. Eighty healthy subjects were randomly assigned to receive either placebo or UP446 250mg**,** in identically appearing capsules**,** once daily for 60 days. Subjects were sex-matched and recruited from ages 40 to 75. Subjects taking NSAIDs underwent a two-week washout period before beginning the baseline visit. Physical activity was not restricted nor were the subjects given any advice as to diet. Safety was measured by the incidence of treatment emergent of adverse events and laboratory abnormalities. Subjects were free to withdraw from the trial at any time for any reason. An independent review board approved the protocol and all patients were required to provide written informed consent prior to enrollment and administration of any medication.

Statistical analysis

Power calculations were made for each phase of the present study. In analyzing blood chemistry data it was determined that 80% power to detect a 10% difference in change between treatment and control groups, given a 20% coefficient of variation**,** could be achieved using 8 animals per group; using 10 animals per group provided an estimated power of 89%. For the human safety data it was determined that a sample size of N=40 per group would provide at least 80% power to detect a relative risk of 1.5 or greater compared to placebo.

Because of the safety nature of the present study, it was determined that avoiding a Type II error (missing an effect that is actually present) was the most important goal. To this end**,** the

alpha level was set at 0.05 for all comparisons, despite the many comparisons being made. This decision served to increase power to detect potential safety concerns, and investigators evaluated the clinical significance of any significant changes observed.

Body weight data was analyzed with a repeated measures analysis of variance (ANOVA) for both the acute (2-week) and chronic (13-week) treated mice. This analysis was also used to detect differences in body weight patterns for treated aged rats compared to untreated aged rats**,** as well as for treated young rats compared to untreated young rats.

Laboratory results (hematological parameters and serum biochemistry results) were compared between groups (treated and untreated) using Student's *t* statistic. Any differences between groups**,** as well as observed values which fell outside the expected reference range, were evaluated by the lead investigators.

RESULTS

Characterization of Isolated and Purified Extracts

For the *S. baicalensis* standardized extract using HPLC/PDA/MS**,** as well as ^{1}H NMR analysis, baicalin, wogonin-7-glucuronide, oroxylin A 7-glucuronide, baicalein, wogonin, chrysin-7-glucuronide, 5-methyl-wogonin-7-glucuronide, scutellarin, norwogonin7-glucuronide, chrysin and oroxylin were identified. Using ^{13}C NMR, two structures were identified from the *A. catechu* standardized extract as catechin and epicatechin (Table 1). Three different extractions and preparations of the *S. baicalensis* and *A. catechu* varied by no more than 10% for each component. A standardized ratio of 4:1 extracts for *S. baicalensis* to *A. catechu* were mixed to optimize the inhibition of cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) enzymes (data not shown). The final content for mixed extract by HPLC analysis and UV detection is shown in Figure 2.

Cell toxicity

We used THP-1 (ATCC # TIB-202), a human immortalized monocyte, to assess cytotoxicity in a LDH assay (Schafer et al., 1997). When the cells were grown to confluent monolayers and then exposed to increasing levels of non-steroidal anti-inflammatory drugs (NSAIDS), celecoxib and UP446, only indomethacin and celecoxib showed significant cytotoxicity above 50 μg/ml inducing apoptosis. UP446 showed low cytotoxicity at the highest test concentration, 100μg/ml (Figure 3). Observations of cell morphology also showed prominent "blebbing" indicative of apoptosis for indomethacin**,** and celecoxib at concentrations as low as 2 μg/ml (data not shown).

In vivo study observations

In all animal studies, food and water consumption, activity, and behavior remained constant and normal. There was no morbidity of animals throughout the entire study.

Body weight trends

Weight measurements for the acute 2-wk, and chronic 13-wk, treated mice were similar to weights observed for the placebo administered controls (Figures 4AB). Since baicalin and catechin, the active ingredients in UP446, have been formulated for use in aging human populations suffering from osteoarthritis, the mixture was tested in aged F344 rats (18 months) versus young F344 rats (6 month) to assess gastric toxicity. Each group was weighed weekly. Test animals were dosed with UP446 at 34 mg/kg/d after three wks acclimation to facilities. Average body weights for the older and younger animals fed UP446 at 34 mg/kg/d were statistically indistinguishable from controls (Figure 5AB).

Hematology

Group mean values for hematology for ICR mice fed UP446 for the acute (2000 mg/kg/d) and subchronic (50, 250, 500 mg/kg/d) toxicity studies are shown in Table 2. Statistically significant group mean values for hematology for old and young control**,** as well as young and aged F344 male rats fed 34 mg/kg/d of UP446, are shown in Table 3. Only minor differences were observed between groups with most values falling within reference ranges**,** except for the 50 mg/kg/d dosage group in males.

Serum biochemistry

Group mean blood chemistry values for ICR mice fed UP446 in subchronic and acute toxicity studies are shown in Table 4. Blood chemistry results for the young F344 control, aged F344 control and aged F344 male rats fed UP446 at 34 mg/kg/d are shown in Table 5. Comparable levels for all analytes were seen in the young and aged controls**,** as well as the young versus old animals fed 34mg/kg/d of UP446 except for potassium levels.

Histology

Liver, kidney, duodenum and stomach tissue samples of ICR mice and F344 rats fed UP446 were compared to the untreated animals in the acute and chronic dosing regimes for changes in histology. Histological findings showed that there were no changes in liver, duodenum or stomach tissues. The pathologist's report showed, however, that aged rats, whether untreated or treated with UP446, showed mild to moderate glomerulonephropathy which was interpreted to be consistent with the normal aging process in these animals and not due to the test agent. No other histological differences between treated and untreated animals were seen.

Cytochrome P450 inhibition

The combined extract was examined in a CYP450 inhibition prescreen assay (mean of 2 replicates per assay). A critical function of CYP450 enzymes is detoxification of drugs. Significant inhibition would indicate the potential for drug interactions. The UP446 product showed only moderate inhibition of CYP1A2 (23%) at a 10μM concentration, and showed low inhibition for all other CYP isoforms (11-16%) versus the controls furafylline, sulfaphenazole,

tranylcypromine, quinidine, and ketoconazole which showed 90-100% inhibition of CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 respectively.

AMES Test

The combined UP446 extract caused no increase in the number of spontaneous revertants at any dose level in any of the following strains: TA98, TA100, TA1535, TA1537 and TA102. An example of this is shown for strain TA1535 (Figure 6). In the presence of metabolic activation (+S9), moderate to marked signs of cytotoxicity were noted in the range of 79-5000 μg/plate in 3 strains: TA100, TA1535 and TA1537. Under both experimental conditions (±S9), there were no biologically significant increases in mutagenicity in any of the five tester strains (data not shown).

Human clinical study

A pilot human safety trial was conducted at Georgetown University in healthy adults. Of those completing the study, 29 received 250 mg per day of the combined extract and 39 received placebo once daily for 60 days. Symptomatic adverse events were few, mild and comparable in treated and placebo groups (Table 6). The observed relative risk was 1.44 with a 95% confidence interval ranging from 0.83 to 2.49. Because the confidence interval includes 1, this relative risk ratio is non-significant when looking at total adverse events.

Subjects were also evaluated for changes in plasma biochemistry from baseline to post-treatment. No mean values for any group fell outside of the clinically acceptable ranges for either group. Thus, only changes in marker values were presented to look for trends that might

indicate safety concerns. These data are presented in Table 7. Investigation of Table 7 reveals that there are minor systematic changes in blood chemistry due to exposure to UP446 which did not fall outside reference ranges. Both treatment and placebo groups showed a significant decrease in Total Cholesterol to HDL Cholesterol; this difference appeared to be due to an increase in HDL Cholesterol, which was observed in both groups, but only reached statistical significance in the placebo group. The placebo group showed a significant increase in MCHC, and the treatment group showed a significant decrease in Potassium. However, as mentioned above, none of these changes fell out of the clinical ranges for the marker in question, and the changes appear to be largely random in nature. For no marker was there a statistically greater change for one group compared to the other.

DISCUSSION

In the present study, we analyzed the toxicity of a combination extract, UP446, with over 62% baicalin and 10% (+)-catechin (Figure 1) content, as well as other related flavonoids: wogon-7-glucuronide at ~6.7%, and oroxylin A 7-glucuronide baicalein, wogonin, chrysin-7-glucuronide, 5-methyl-wogonin-7-glucuronide, scutellarin, norwogonin, chrysin oroxylin A, all present under 2% or in trace amounts (Table 1; Figure 2). There is also 3% unidentified material**,** along with 5% moisture and 6% maltodextran as an excipient. The content of any one flavonoid did not vary by more than 10% in 3 different batches.

Extracts of *S. baicalenis*, *A. catechu* and other plant flavonoids have been shown to be safe in animals in dosing far exceeding those that would be given humans primarily in LD_{50} studies (Oyo Yakuri, 1982; Chang and But, 1987; Yin et al. 1991; Ksrnam, 1990; NIIRDN Drugs, 1990; Zhang et al., 1998 Galati and O'Brien, 2004; Isbrucker et al., 2006). To our knowledge, the combination of *S. baicalensis* and *A. catechu* defined extracts**,** with the proportion of flavonoids here, has never been tested. This knowledge is required before giving this specially formulated mixture to patients especially as a prescription medical food product for osteoarthritis.

NSAIDs have been shown to induce apoptosis in different human cancer cell lines (Piazza et al., 1995; Elder et al., 1996; Shiff et al., 1996), but also in monocytes, macrophages and gastric mucosal cells (Simmons et al., 1999; Tsutsumi et al., 2004; Tanaka et al., 2005). In our studies, cell toxicity results in a monocytic cell line, THP-1, showed that UP446 exhibited low cytotoxicity at the highest concentration tested, while significantly toxic effects including

apoptosis were observed at same concentration from indomethacin and celecoxib (Figure 3). Continual exposure to NSAIDs, including selective COX-2 inhibitors**,** has been proposed to generate new COX-2 genetic variants over time that could complicate inflammatory conditions such as osteoarthritis (Simmons et al., 1999).

Oral dosing of UP446 was well-tolerated in 5-6 wk old ICR mice in 2-wk acute and 13-wk subchronic toxicity studies. Similar to other oral dosing studies (Kiwaki et al., 1989ab), there was no adverse effect on overall health or weight at doses in mice up to and including 2 g/kg/d (Figure 4). Unlike the LD_{50} data found for (+)-catechin at 2.17 g/kg/d found previously in mice (Ksrnam, 1990), however, no deaths were recorded in this study even at the highest dose of 2 g/kg/d for UP446. The doses of 50, 250, 500, and 2000 mg/kg/d given in mice are equivalent to 250, 1250, 2500 and 10,000 mg daily dosages in humans with average body weight of 70 kg using standard body surface area conversions (Freireich et al., 1966). Only the 50 and 250 mg/kg/d doses fall within the recommended dosages to humans for UP446 in both the supplement form and the prescribed medical food suggesting from the results that humans should also tolerate the combined extract.

Serology values for male ICR mice administered 50 mg/kg/d were low for WBC, RBC, Hb and MCH values (Table 2). These observations were not seen in the other dose groups in male mice**,** except for the RBC value in the 2000 mg/kg/d male group. All female ICR mice were generally within the noted reference ranges**,** except for the MCV value at 2000 mg/kg/d. Hematology for young rats at 0 and 34 mg/kg/d was normal. Older rats on placebo and 34 mg/kg/d both showed lower WBC counts suggesting this effect was unrelated to UP446 (Table

3). Platelet counting was performed using the RBC/platelet ratio method (*International Council for Standardization in Haematology Expert Panel on Cytometry; International Society of Laboratory Hematology Task Force on Platelet Counting*, 2001). This method is particularly sensitive to hemolysis or clotting. The pathology report noted that when platelet counts were low, that hemolysis was present and hence, the counts were inaccurate in ICR mouse analysis. This same phenomenon was not observed in the rat samples. Sample handling inconsistencies and low volumes of serum from mice compared to those needed for processing may have contributed to the variable values seen for the 50 mg/kg/d UP446 dosed to male mice, as well as the platelet anomalies.

Blood chemistries in male and female ICR mice fell within the reference ranges with the exceptions of slightly elevated alanine aminotransferase (ALT) in the 2000 mg/kg/d dosing in males and slightly depressed alkaline phosphatase (ALK Phos) in the 50 mg/kg/d male and female groups and the 250 mg/kg/d in males (Table 4). These values are not dramatically outside the reference ranges, are not reproducible at different doses and could represent normal error ranges in the clinical assays.

Overall health and weight in young and old rats was unaffected by UP446 (Figure 5). The rat blood chemistries fell within the reference ranges for the placebo and 34 mg/kg/d dosage groups for the young and old rats. However, all rat blood samples exhibited very high, potentially lethal potassium levels (Table 5). Ciandanol and its metabolites, (+)-catechin related compounds, have been implicated in at least six cases of hemolysis in humans (Salama and Mueller-Eckhardt, 1987). *In vitro* hemolysis, however, can also occur in a blood sample owing to prolonged storage

or storage in incorrect conditions (Cox, 1981). The pathology report noted that all rat samples, even the controls not dosed with UP446, were hemolyzed upon arrival. Rather than this data representing a hyperkalemic state caused by UP446, elevated potassium levels in all rat sera were due to hemolysis from delayed sample processing. Therefore, hemolysis was not attributed to a specific effect of UP446 on the kidney. Potassium levels in all mice sera were within in the reference range (Table 4).

NSAIDS typically affect two major organs; kidney and stomach, with the stomach showing the greatest sensitivity due to COX-1 inhibition (Griffen et al., 1991). Protection of gastric mucosa requires the constant production of prostaglandin E_2 (PGE_2) and prostacyclin I_2 (PGI_2) (Wallace and Granger, 1996). Arthritic medication, in particular, presents a problem of toxicity for physicians and their patients due to their inhibition of PGE_2 and PGI_2 generation which results in varying degrees of ulceration depending on the agent used (Whittle, 1986). Histological examination of liver, kidney, stomach and duodenum revealed no abnormalities in any of the ICR mice at any dosage in the acute and subchronic studies. Mild vacuolar change and hyperplasia of the liver tissue was consistently noted in both treated and untreated mice. Histological results from Fischer 344 rats exposed to UP446 were more interesting due to the results found for the stomach and duodenum.

Rats represent a species sensitive to the adverse effects of traditional NSAIDs forming gastrointestinal lesions dosed with as little ibuprofen as 2 mg/kg/d, equivalent to approximately 140 mg/d in humans (Elliott et al., 1988). The pathology report noted that there were no mucosal or duodenal lesions seen in any rats dosed with the human equivalent of UP446 of 370 mg/d over

a 9 week period. These findings suggest that the mixed extract containing high levels of baicalin and (+)-catechin is well-tolerated by the gastrointestinal mucosa in rats and will presumably be well-tolerated in humans as well.

Other flavonoids have also been shown to either be gastric protective or have been used for the treatment of gastric ulcerations (Parmar and Ghosh, 1973). For example, meciadanol (O-methyl-3(+)-catechin), a (+)-catechin related molecule, was used to treat post-operative, stress ulcerations in humans (Kitler et al., 1990). Mild vacuolar change of hepatocytes present in the rat livers are similar to the control animals and thus considered to be within normal limits. Mild to moderate glomerulonephropathy of kidney tissue was consistent with chronic progressive renal disease and present in both the control and 34 mg/kg/d dosed aged rats. Therefore, this change could not be attributed to UP446. Effects on overall health, weight, blood chemistry and serology and tissue histology are only part of a proper toxicological study. It is also important to know how compounds interact with drug metabolizing enzymes.

An extensive review of flavonoid interactions with CYP450 isoenzymes has been published (Hodek et al., 2002). Many different flavonoids, including those related to baicalin and (+)-catechin, interact particularly with CYP3A4. Kim *et al.* (2002) demonstrated, for example, that 122.4 μg/ml of an aqueous *S. baicalensis* extract inhibited CYP3A4 in rat liver microsomes. Human CYP activity, however, was not affected by the same extract (Kim et al., 2001). Baicalein, found in low concentrations in UP446, has been shown to inhibit both CYP1A1 and CYP1B1 thereby modulating the formation of carcinogen-DNA adducts (Chan et al., 2002), whereas green tea extracts containing complex catechins like ECGC have been shown to activate

CYP1A1 and perhaps aid in the prevention of liver cancer (Liu et al., 2003). Structural binding studies with baicalein have shown that it may reduce the activity of CYP19 aromatase**,** thus suggesting an anti-estrogenic activity (Kao et al., 1998). In order to assure safety of UP446 containing baicalin and (+)-catechin, when consumed with medications, a CYP450 isoenzyme inhibition study was undertaken.

Most clinically important drug inhibitors have K_is of <10μM. Therefore, if a compound inhibits the CYP450 isoenzymes to ≥ 50%, it is recommended that the samples may have a major drug interaction and should be further characterized by determination of an IC_{50} or a K_i. This could then provide a preliminary assessment of potential drug interaction as described by the Cheng-Prusoff Equation (Craig, 1993). In our assay, the use of UP446 at a concentration of approximately 10μM showed moderate inhibition (23%) of CYP1A2 and only a slight inhibition of CYP3A4 (11%), the principal metabolizing enzyme of 50% of all pharmaceutical drugs (Michalets, 1998; Thummel and Wilkinson, 1998). Similar inhibition findings were noted in each study for CYP2C9, CYP2C19 and CYP2D6 (11-16%). Because UP446 never inhibited any one of the five principle drug metabolizing enzymes to 50%, the extract does not require further determination of an IC_{50} or a K_i and is judged not have a pronounced inhibitory effect on drug metabolizing enzymes. Therefore, UP446, by this analysis, does not have major drug interactions and should be safe take with most medications.

An AMES test detects the mutagenic potential of compounds that functionally reverse mutations present in strains of *S. typhimurium*, thus restoring the capability to synthesize histidine (*his*- to *his*+ genotype and phenotype), an essential amino acid. Both baicalin and

baicalein were tested for their antimutagenic potential in the *S. typhimurium* strain TA100 (Ohtusuka et al., 1995). Both were able to inhibit the mutagenicity induced by 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2), a known carcinogen. Wogonin slightly inhibited the AF-2 induced mutagenesis when simultaneously incubated with the carcinogen, but appeared to activate mutagenesis to a moderate level when AF-2 was added after wogonin exposure in the same assay. This suggests mutagenic potential for wogonin. A methanolic extract of *S. baicalensis* was also shown to be antimutagenic potential on *S. typhimurium* strains TA98 and TA100 (Wozniak et al., 2004). Even with this evidence there was a need to evaluate the mutagenesis on the combined UP446 extract to assure safety, especially since wogonin exists at ~1% in UP446 was shown to induce mutagenicity in other assays (see above).

The mutagenic potential of UP446 was rigorously assessed in a plate incorporation method with and without metabolic activation on *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA102 (Maron and Ames, 1983; Gatehouse et al., 1994). The mixed extract caused no mutagenicity as judged by a reduction of the bacterial background lawn or decrease in the number of spontaneous revertants at any dose level and in any of the five strains. In the presence of liver enzymes for metabolic activation, the extract did not increase the incidence of revertants, but was cytotoxic in 3 strains of *S. typhimurium*, TA98, TA100 and TA1535. Baicalin and related Free-B-ring flavonoids have antimicrobial activity and antiviral activity which may account for the cytotoxicity observed in certain strains of *S. tymphimurium* (Afolayan and Meyer, 1997; Meyer et al., 1997). This result strongly suggests that the mixture of flavonoids in UP446 does not have a high mutagenic potential and is presumed safe for human consumption. This was tested in a low dose experiment in a pilot human safety trial.

In the pilot human safety trial described herein, subjects dosed with 250 mg of combined extract per day did not develop any hematological or plasma biochemical abnormalities (Table 7). All markers measured remained in clinically acceptable ranges for the duration of the study, and any statistically significant changes appeared to be largely arbitrary rather than systematic and indicative of potential concern. One potentially interesting change was the statistically significant decrease in the ratio of total cholesterol to HDL cholesterol. However, as this change in ratio was observed in both the treatment and placebo group, little inference can be drawn from this result and further research on this topic is warranted.

Clinical adverse events were mild and comparable to placebo (Table 6). The observed relative risk ratio of 1.44 for total adverse events in the treated group compared to controls was non-significant (95% CI 0.83 – 2.49). Also notably absent were any reported gastrointestinal adverse events in the treatment group. This finding is important as a primary side effect of NSAIDs is gastrointestinal bleeding or other difficulties, so for treatment of osteoarthritis an absence of these types of adverse events is an important consideration. The level of adverse events in the UP446 exposed and the placebo groups were not elevated when compared to other clinicals published for anti-inflammatory agents. Kivitz et al. (2001), for example, tested celecoxib and naproxen against placebo for safety and efficacy reporting overall adverse events rates in greater than 50% in call groups tested. Thus, the present study suggests that the mixture of flavonoids in UP446 is safe for human consumption, both overall and, importantly, with respect to gastrointestinal adverse events, at the levels tested.

CONCLUSION

Our results suggest that the mixed extract containing baicalin and catechin is likely to have a favorable toxicity profile and deserves further investigation as a potential agent for the dietary metabolic management of osteoarthritis.

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Table 1

Chemical Composition of UP446

Active Compounds	Composition
***S. baicalensis* Extract**	
Baicalin	62.5
Wogon-7-glucuronide	6.7
Oroxylin A 7-glucuronide	2.0
Baicalein	1.5
Wogonin	1.1
Chrysin-7-glucuronide	0.8
5-methyl-wogonin-7-glucuronide	0.5
Scutellarin	0.3
Norwogonin	0.3
Chrysin	<0.2
Oroxylin A	<0.2
Total Free-B-ring flavonoids	75.7±10%
***A. catechu* Extract**	
Catechin	9.9
Epicatechin	0.4
Total Flavans	10.3±10%
Total Active Ingredients	86±10%*

*The UP446 combination, standardized extract also contained approximately 3% unidentified content, 5% moisture, and 6% added excipient (maltodextran) to control flow characteristics of the final composition in the powdered form. Each component in the mixture varied by not more than 10%.

Table 2

Hematological Parameters for ICR Mice Exposed to UP446.

	Males					Females					Ref. [+ϕ]
Dose Level (mg/kg/day)	**0**	**50**	**250**	**500**	**2000**	**0**	**50**	**250**	**500**	**2000**	**-**
WBC(10^3/ul)[ψ]	3.8 (0.4[†])	**1.0**[γ] (0.3)	5.0 (0.5)	5.5 (0.5)	2.2 (0.2)	2.6 (0.3)	2.1 (0.4)	2.4 (0.3)	3.4 (0.4)	4.1 (0.2)	1.8-13.1
RBC(10^6/ul)	7.0 (0.3)	**3.3** **(0.3)**	8.7 (0.6)	7.0 (0.4)	**3.3** **(0.2)**	7.0 (0.7)	7.2 (0.7)	5.9 (0.5)	7.1 (0.5)	5.5 (0.6)	5.5-10.5
Hb (g/dl)	9.3 (0.4)	**2.1** **(0.5)**	14.1 (0.9)	11.6 (1.0)	13.3 (1.5)	9.5 (0.7)	8.9 (0.8)	7.4 (0.4)	11.8 (0.7)	7.3 (0.8)	7.0-12.5
MCV (fl)	54.1 (7.3)	60.9 (7.7)	45.9 (5.2)	50.5 (3.3)	58.5 (4.4)	51.8 (5.0)	53.2 (6.0)	51.5 (5.1)	48.6 (5.5)	**70.5** **(5.0)**	45.0-60.6
MCH (pg)	14.0 (1.3)	**8.9** **(0.7)**	16.3 (1.0)	16.5 (0.7)	17.9 (1.8)	12.9 (1.1)	11.4 (1.0)	11.6 (0.9)	15.9 (1.1)	15.9 (1.5)	11.4-19.6
PLT (10^3/ul)	343 (72)	480 (58)	397 (50)	**42** **(10)**	530 (49)	**53** **(9)**	371 (49)	513 (83)	**60** **(13)**	**95** **(23)**	164-1411

[ψ] WBC, white blood cell count; RBC, red blood cell count; Hb, hemoglobin; MCV, mean cell volume; MCH, mean cell hemoglobin; PLT, platelet count.

[†] Values in apprentices are standard deviations.

[+] Mid Atlantic Regionl Laboratory, 10 week old male and female mice.
Mitruka BM and Rawnsley HM (1977) *Clinical and Hematological Reference Values of Normal Experimental Animals,* Masson Publishing, New York, NY.

[ϕ] Ranges represent an average of the male and female referenced ranges.

[γ] Emboldened observations lie outside reference ranges.

Table 3

Hematological Parameters for F344 Rats Exposed to UP446.

	Young		Aged		Ref.[+*ϕ]
Dose Level (mg/kg/day)	**0mg/kg**	**34mg/kg**	**0mg/kg**	**34mg/kg**	
WBC (10^3/ul)	3.6 (0.5[†])	4.2 (0.2)	**1.5 (0.5)**[γ]	**1.7 (0.4)**	3.4-10.2
RBC (10^6/ul)	8.9 (0.8)	7.9 (0.2)	5.8 (1.2)	6.2 (0.7)	6.0-9.8
Hb (g/dl)	18.2 (2.1)	15.6 (1.1)	13.4 (2.6)	12.5 (2.4)	12.4-15.8
MCV (fl)	59.5 (2.4)	61.4 (3.0)	62.5 (13.4)	69.5 (10.3)	49.8-57.8
MCH (pg)	21.1 (2.0)	20.8 (1.1)	**22.2 (2.6)**	21.0 (1.7)	19.3-21.6
PLT (10^3/ul)	357 (41)	297 (15)	314 (55)	208 (45)	150-694

[†] Values in apprentices are standard deviations.

[+] Wistar albino strain, male, weighing 180-250 g as a reference.

***** Mitruka BM and Rawnsley HM (1977) Clinical and Hematological Reference Values of Normal Experimental Animals, Masson Publishing, New York, NY.

[ϕ] Harlan Sprague Dawley, Inc. Standard Values.

[γ] Emboldened observations lie outside reference ranges.

Table 4

Plasma Biochemistry Parameters of ICR Mice Exposed to UP446

	Males					Females					Ref. [+]
Dose Level (mg/kg/day)	0	50	250	500	2000	0	50	250	500	2000	-
[Ψ]ALT (IU/l)	36.1 (7.2[†])	36.0 (5.5)	36.5	35.0	**95.0**	34.3 (4.8)	24.1	25.5	53.2	58.0	24-81
Bil (mg/dl)	0.3 (0.10)	0.3 (0.10)	0.3	0.3	0.2	0.4 (0.11)	0.4	0.3	0.4	0.2	0.1-0.4
ALK Phos	17.1 (3.7)	10.5 (2.7)	10.1	30.5	61.0	13.9 (2.3)	10.0	20.5	11.4	69.4	11-117
TP (g/dl)	4.8 (0.3)	4.6 (0.7)	4.8	5.0	5.0	4.9 (0.8)	5.1	4.9	4.7	4.8	3.5-7.2
ALB (g/dl)	2.5 (0.3)	2.5 (0.3)	2.6	2.4	3.0	3.0 (0.3)	3.3	3.0	2.9	3.0	2.4-3.6
GLB (g/dl)	2.3 (0.3)	2.1 (0.3)	2.2	2.6	2.0	1.9 (0.3)	1.8	1.9	1.9	1.8	1.6-2.9
TC (mg/dl)	119.2 (34.3)	115.5 (18.9)	125.0	117.5	133.8	84.9 (19.0)	84.6	79.0	79.0	94.0	50-250
BUN(mg/dl)	23.5 (5.1)	26.0 (4.8)	24.0	20.0	25.2	34.1 (3.2)	36.5	35.1	27.5	24.7	18-37
CRN(mg/dl)	0.6 (0.18)	0.5 (0.20)	0.7	0.6	0.8	0.8 (0.12)	1.0	0.5	0.5	0.8	0.3-1.0
P (mg/dl)	6.1 (0.6)	5.7 (1.0)	5.8	5.5	9.5	6.9 (1.1)	6.9	6.8	6.8	7.6	5.3-11.4
Ca (mg/dl)	7.9 (0.4)	7.8 (0.8)	7.8	8.0	8.4	7.9 (0.7)	7.8	7.9	7.9	8.4	3.2-8.5
Gluc(mg/dl)	222.7 (28.2)	225.0 (20.5)	230.5	251.0	187.0	182.0 (22.3)	124.0	204.6	214.2	194.6	124-211
Na (mEQ/l)	147.5 (22.8)	141.0 (15.8)	143.0	143.5	150.4	140.4 (18.5)	140.0	142.1	139.7	144.4	140-169
K (mg/dl)	5.6 (0.9)	4.8 (0.7)	4.5	4.4	5.6	5.0 (0.6)	5.9	4.3	4.6	7.3	3.9-8.8
Cl (mEQ/l)	122.5 (9.4)	111.0 (11.1)	111.0	112.5	114.4	109.9 (19.1)	111.0	109.7	109.1	108.2	100-123

[Ψ] ALT, alanine transferase; Bil, total bilirubin; ALK Phos, alkaline phosphatase; TP, total protein; ALB, albumin; GLB, globulin; TC, total cholesterol; BUN, urea nitrogen; CRN, creatinine; P, phosphorous; Ca, calcium; Gluc, glucose; Na, sodium; K, potassium; Cl, chloride.

[†] Values in apprentices are standard deviations.

***** Mid-Atlantic Regional Laboratory, 10 week old male and female mice.

[+] Suckow, Mark A., Danneman, Peggy, Brayton, Cory, *The Laboratory Mouse, a volume in the Laboratory Animal Pocket Reference Series* (Boca Raton: CRC Press, 2001); Gad A and Chengelis CP (1992) *Animal Models in Toxicology*, (Dekker Incorporated, Marcel).

Table 5

Plasma Biochemistry Parameters for F344 Rats Exposed to UP446.

	Young		Aged		Ref. [+*]
Dose Level (mg/kg/day)	**0mg/kg**	**34mg/kg**	**0mg/kg**	**34mg/kg**	**-**
ALT (IU/l)	100.8	72.7	127.7	74.5	59-91
Bil (mg/dl)	0.2	0.2	0.3	0.4	0.1-0.5
ALK Phos	119.8	152.3	134.8	74.8	75-355
TP (g/dl)	7.2	6.6	7.5	7.6	6.5-8.0
ALB (g/dl)	4.2	3.6	3.8	4.1	3.4-4.6
GLB (g/dl)	2.7	3.6	3.7	3.5	2.4-3.6
TC (mg/dl)	96.3	119.7	200.7	177.7	74-126
BUN (mg/dl)	22.0	19.3	26.3	24.5	16-27
CRN (mg/dl)	1.0	0.7	1.5	1.3	0.4-1.5
P (mg/dl)	11.0	6.2	15.7	8.5	6.9-8.5
Ca (mg/dl)	9.3	9.5	9.2	9.2	9.1-11.4
Gluc (mg/dl)	103.2	110.3	126.2	104.0	88-182
Na (mEQ/l)	130.0	126.7	134.8	127.2	127-150
K (mg/dl)	**30.0**	**27.6**	**20.7**	**43.1**	4.9-6.0
Cl (mEQ/l)	96.3	97.7	105.0	99.3	92-126

[+] Harlan Sprague Dawley, Inc., 12-wk old F-344 male and female rats as a reference.

[*] NIEHS, (1985) *A summary of control values for F344 rats and B6CF1 mice in 13 wk*

subchronic studies, Program Resources, Inc. Research Triangle Park, NC; Burns KF, Timmons EH and Poiley SM (1971) "Serum chemistry and hematological values for axenic (germfree) and environmentally associated inbred rats," *Lab. Anim. Sci*., **21:** 415.

Table 6

Adverse Events Observed in Humans Administered UP446

UP446 (N=29)		Placebo (N=39)	
Adverse Event	**#**	**Adverse Event**	**#**
CNS*	9	CNS*	6
Rash	1	GI[¥]	5
Heartburn	1	Pruitis	1
Flu Symptoms	1	Myalgias	1
Sinus Infection	1	Prostate	1
Myalgias	1		
Water Retention	1		

* Central Nervous System disturbances (CNS): Drownisess, irritability, dizziness, insomnia, fatigue, nervousness, disturbing dreams

[¥] Stomach pain, irritable colon, nausea

Table 7

Changes in Hematological and Plasma Biochemistry Parameters[Ψ] in Humans Exposed to UP446

Variable	Treatment (N=29)		Placebo (N=39)		P value Between Groups
	Mean diff	P value	Mean diff	P value	
ALBUMIN	-.34	.31	-.02	.51	.88
AG RATIO	-.01	.79	-.01	.74	.42
ALK PHOS	.38	.79	.90	.57	.64
ALT (SGPT)	-.14	.93	.56	.55	.20
AST (SGOT)	-.62	.54	.15	.77	.93
BASOPHILS	-.10	.11	.15	.24	.16
BASO, ABS	-6.2	.08	1.9	.53	.38
BILI, TOTal	.04	.18	-.02	.50	.45
BUN/CREAT RATIO	-.17	.84	-.44	.46	.15
CALCIUM	-.04	.43	.06	.18	.96
CARB DIOX	.10	.82	-.13	.76	.42
CARDIO CRP	.003	.99	.36	.60	.79
CHLORIDE	-.59	.12	-.05	.86	.54
CHOL/HDLC RATIO	-.30	**.01***	-.33	**.01**	.21
CHOL, TOT	-3.3	.49	.59	.86	.69
CREATININE	.21	.33	.19	.31	.38
EOSINOPHIL	-.06	.74	-.34	.06	.59
EOSIN ABS	-8.6	.43	-13.9	.14	.57
GLOBULIN	.00	1.0	.03	.47	.98
GLUCOSE	4.0	.44	.49	.83	.76
HDL	2.7	.19	5.0	**.01**	.12
HEMATO	-.80	.07	-.17	.57	.11
HEMOGLOB	-.09	.36	.10	.28	.24
LDL	-7.8	.09	-.44	.86	.69
LYMPHO	-1.4	.24	.42	.56	.96
LYMP, ABS	-32.3	.60	-5.4	.93	.64
MCH	.12	.27	.21	.06	.57
MCHC	.16	.23	.38	**.01**	.19
MCV	-.10	.68	-.41	.13	.52
MONOCYTE	-.30	.17	-.32	.10	.67
MONO, ABS	-12.7	.53	-22.1	.15	.65
NEUT-PHIL	-.03	.99	1.7	.34	.72
NEUT, ABS	280	.17	33.7	.87	.86
PLAT CNT	-2.5	.56	4.3	.61	.90

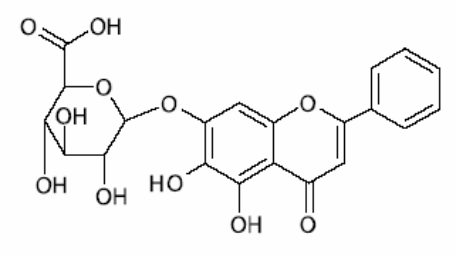
POTASSIUM	-.20	**.01**	-.11	.99	.22
PROT, TOT	-.03	.60	.09	.31	.75
RDW	-.08	.42	.13	.51	.95
RED CELLS	-.05	.14	-.22	.33	.65
SODIUM	-.59	.14	.21	.22	.80
TRIG	3.8	.56	-12.6	.14	.70
TSH	-.10	.65	.02	.17	.24
UREA (BUN)	-.52	.40	-.03	.97	.25
WHITE CELL	.23	.29	.08	.79	.72

$^{\Psi}$ No observed value fell out of the clinically expected range for either group, so only changes in values from baseline to post-treatment are reported.

* Emboldened values represent significant in-group changes.

Figure 1

A.



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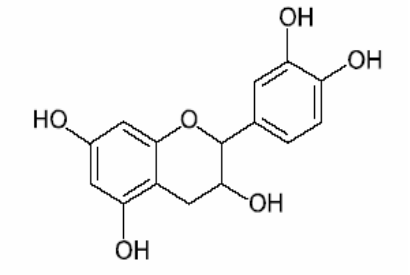

FIG. 1. (A) Baicalin. (B) (+)-Catechin.

Figure 2

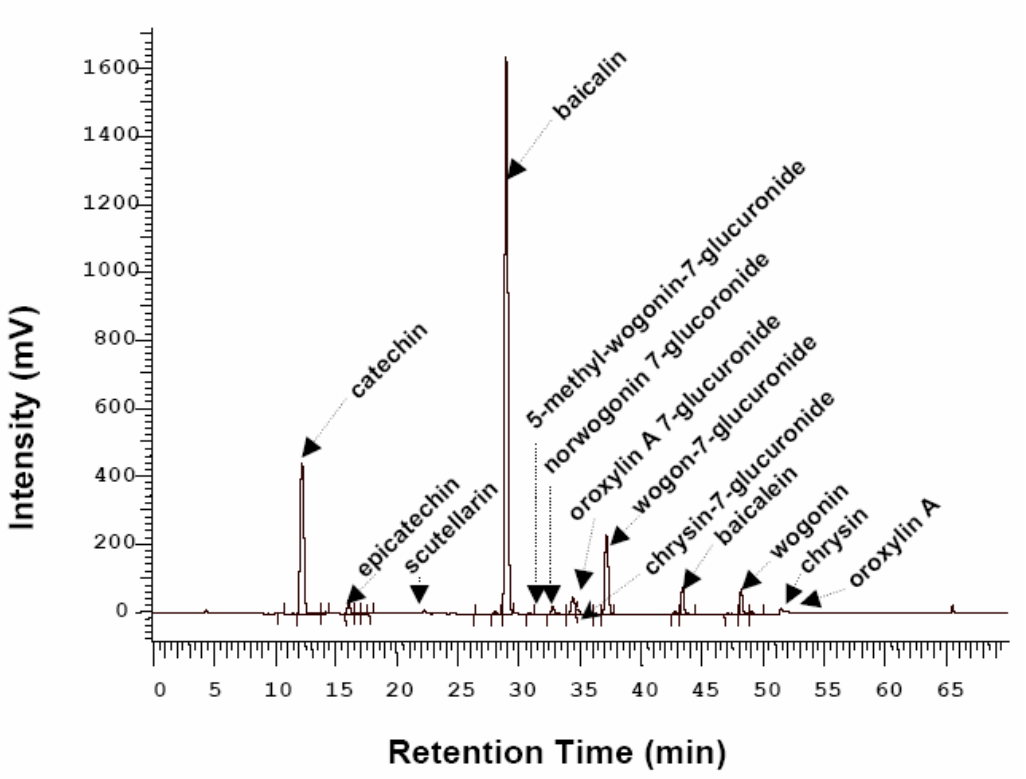

FIG. 2. HPLC purification profile of the combined extract, UP446.

Figure 3

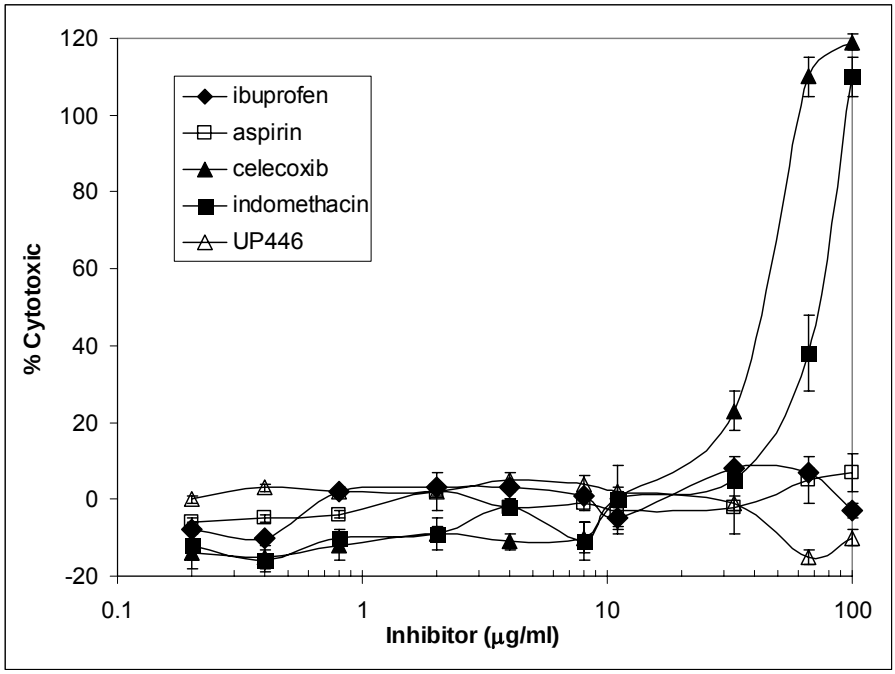

FIG. 3. Cell toxicity in THP-1 monocytes for UP446 (Δ) compared to celecoxib (▲), indomethicin (■), ibuprofen (♦), and aspirin (□) at increasing doses of test material added to the cultures.

Figure 4

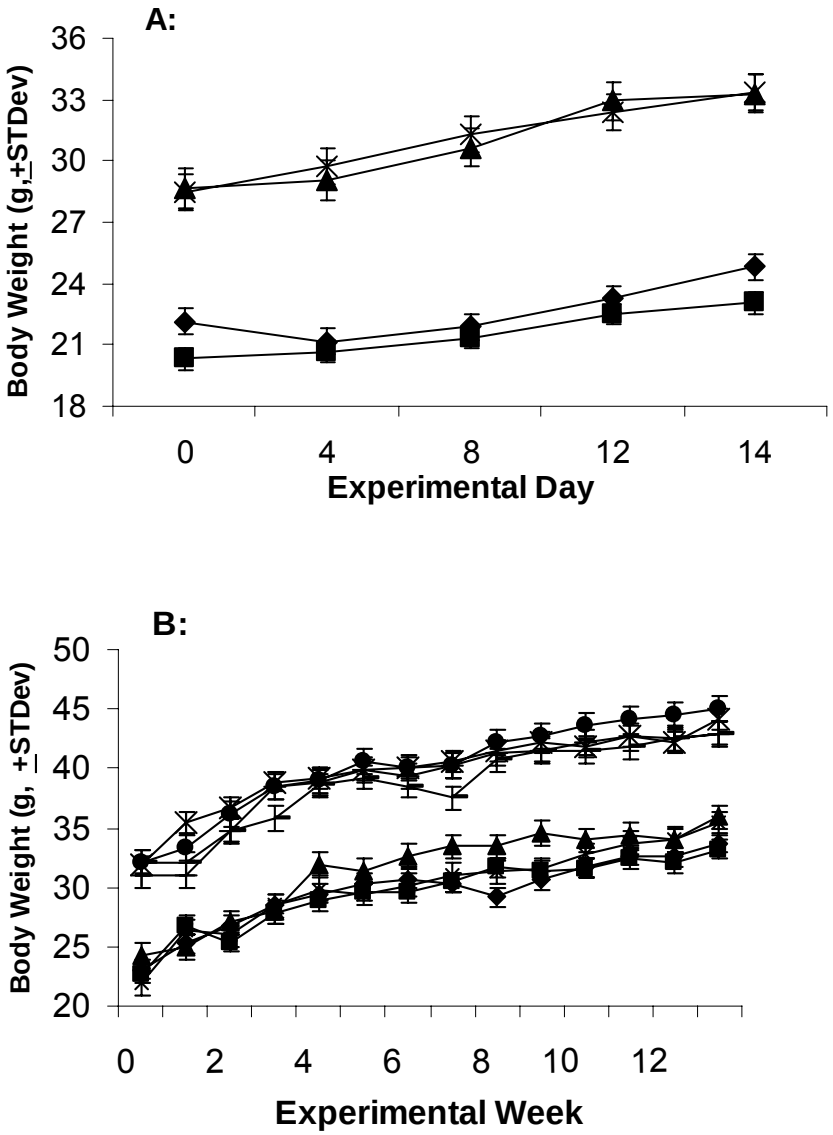

FIG. 4. (A) Body weight trends for 5-6 wk old ICR mice during acute dosing of UP446 at doses of 0 (male mice, ▲; female mice, ■) and 2000 mg/kg/d (male mice, ×; female mice, ♦) for 2-wks. (B) Body weight trends for 5-6 wk old ICR mice during subchronic dosing of UP446 at doses of 0, 50, 250, and 500 mg/kg/d (male mice, ×, –, +, ●; female mice, ▲, ×, ♦, ■) and for 13-wks. Each data point represents the mean±STDev.

Figure 5

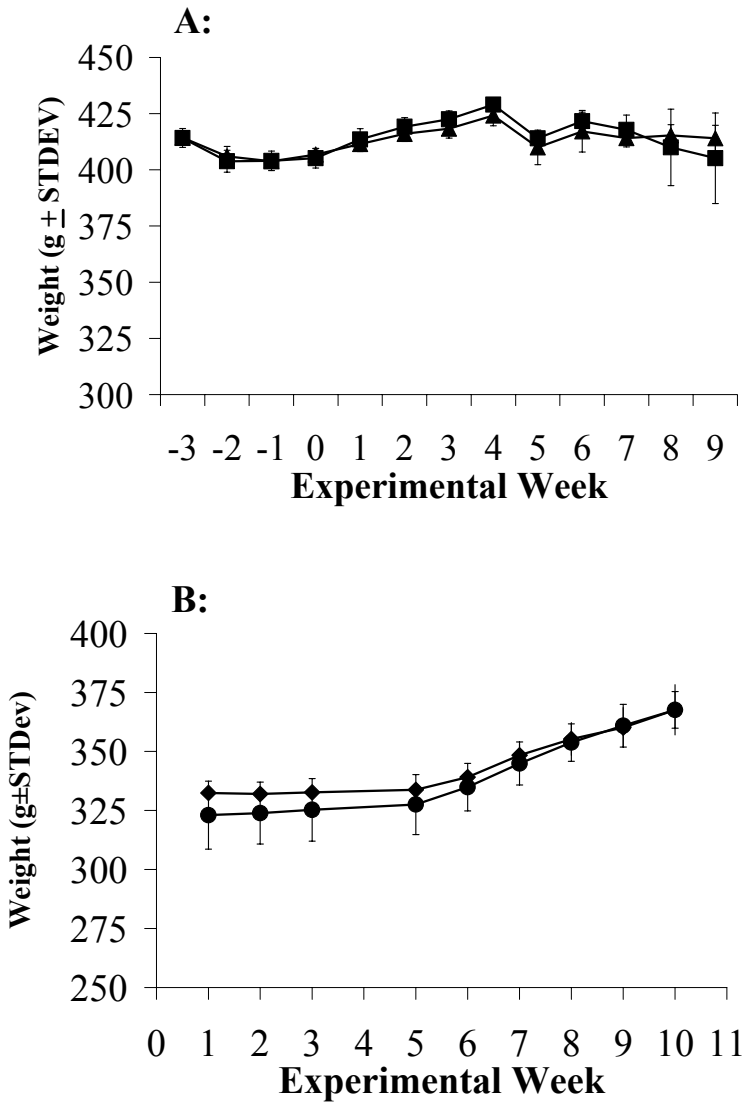

FIG. 5. (A) Body weight trends for 18-month old male F344 rats after a 3-wk acclimatization period and during subchronic dosing of UP446 formulated into NIH31 chow at doses of 0 (■) and 34 mg/kg/d (▲) for 9-wks. (B) Body weight trends for 6-month old male F344 rats during subchronic dosing of UP446 formulated into NIH31 chow at doses of 0 (♦) and 34 mg/kg/d (●) for 10-wks. Each data point represents the mean±STDev (n=12). At wk four, no weights were

taken for the young rats due to a massive snow storm which kept researchers from reaching the lab. Overall health of the colony was unaffected. Each data point represents the mean±STDev.

Figure 6

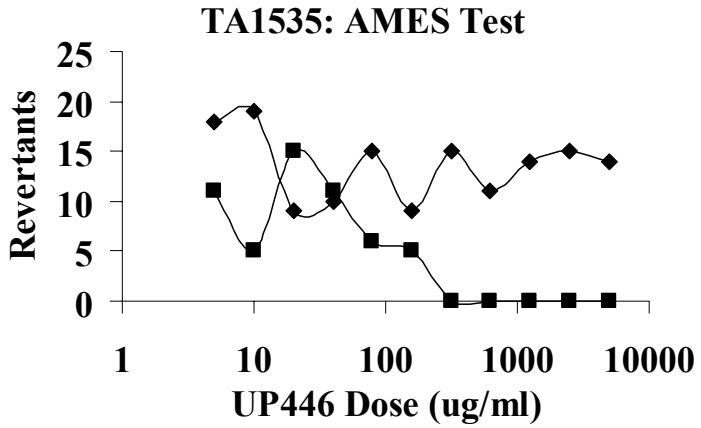

FIG. 6. Reverse histidine mutagenicity of UP446 in *S. typhimurium* strain TA1535 without S9 metabolic activation (♦) and with S9 metabolic activation (■). Doses of UP446 for each test were 5, 10, 20, 40, 79, 157, 313, 625, 1250, 2500 and 5000 μg per plate.