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RESEARCH ARTICLE

A toxicological investigation of a celery seed extract having anti-inflammatory activity

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Abstract
Background and aims An extract of the seed from celery (*Apium graviolens*) (CSE), and fractions thereof, have been found to possess anti-inflammatory activity, gastro-protective activity, and anti-*Helicobacter pylori* activity. In view of the potential for employing these extracts for therapeutic use, toxicological investigations were undertaken with an alcoholic extract (A-CSE) which has previously been shown to have the above pharmacological activities.
Methods A 28-day toxicity study was performed in rats according to Good Laboratory Practice (GLP) conditions. Eighteen adult male and 18 adult female rats were randomly assigned to 3 treatment groups of 6 rats/sex/group and were dosed orally with A-CSE of 0, 150 or 5,000 mg/kg per day. Daily observations of vital signs and body weights were recorded and ophthalmological investigations were performed. At autopsy, the principal organs were weighed and sections collected for histological analysis. Serum and urine samples were collected at termination for routine clinical chemistry. Under non-GLP conditions alpha-2-μ-globulin immunohistochemistry was performed on kidney tissues and hepatic cytochrome P450 protein was determined, as well as, the enzymatic activities of the principal isoforms.
Results All animals survived treatments with no visible or behavioral signs of toxicity being observed during the study. There were no statistically significant differences in body weight gains, body weight gains per day or cumulative absolute body weight gains, for either sex, in any treatment groups when compared with controls. Slightly increased liver weight and liver to body and brain weight ratios were observed in female rats and in liver to body weight ratios in male rats given high dose A-CSE which was a test article effect, but the absence of any microscopic correlates for the liver weight increases suggests that these were not toxicologically significant. Treatment related macroscopic changes were not observed at necropsy and microscopic findings were limited to minimal increases in gastric eosinophils in several male and female rats in the 5,000 mg/kg per day treatment groups. Minimal focal degeneration of renal tubules was observed sporadically in both sexes assigned to all treatment groups including control and was consistent with early spontaneous nephropathy of laboratory rats and thus was not considered to represent a pathologic change associated with the test article. Increased serum globulin and phosphorus levels were observed in male rats given 5,000 mg/kg per day A-CSE and decreased serum triglycerides levels in female animals given 150 or 5,000 mg/kg per day A-CSE. The increase in serum globulin and phosphorus in male animals was small in magnitude and not considered toxicologically significant. The mechanism for the decrease in serum triglycerides in female rats was not apparent. Changes in urinalysis parameters were limited to small decreases in urine pH in female animals in the 150 and 5,000 mg/kg per day groups and were not deemed toxicologically significant. Alpha-2-μ-globulin immunohistochemistry was performed on kidney tissues from all animals and found to be within normal physiologic limits. Minor corneal mineralization occurred in some animals from all treatment groups. Cataracts were observed in one in the control and

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one in an animal that had 5,000 mg/kg per day but since the cataracts occurred in the metabolically inactive region of the lens, these were not considered indicative of test article related lesions. There were no changes in total hepatic microsomal protein or in total cytochrome P450 protein. Although male rats appeared to have to higher levels of total microsomal protein than female rats, there appeared to be no treatment effect in either male or female animals. As regards the activity of the various isoforms tested (CYP2B1/2, CYP1A1/2, CYP3A1/2), with the large range of activities detected for each P450 isoform, no clear change in activity or protein were observed, however, these data were not statistically analyzed.

Conclusions These results suggest that there are no toxicologically significant sub-chronic effects of oral A-CSE in rats. The no adverse effect level for systemic toxicity would appear to be 5,000 mg/kg per day.

Keywords *Apium graveolens* · Celery seed · Toxicology · Cytochrome P450 · Anti-inflammatory activity

Introduction

The seeds and other parts of celery (*Apium graviolens*) have long been employed as herbal medicines (Usher 1974; Blumenthal et al. 1998; Craig 1999, Mdidea 2006). Some of the beneficial properties include anti-bacterial, anti-inflammatory and diuretic effects (Kapoor 1990; Blumenthal et al. 1998). Seeds of the celery plant have been used in Ayurvedic medicine and have been found useful in herbal medicine for the treatment of urinary calculi, gut diseases, relief of flatulence, various painful states, reduction of visceral spasms, and stimulation of the smooth muscle of the womb (Kapoor 1990; Blumenthal et al. 1998; Craig 1999; Mdidea 2006). Some of these effects have been claimed to be related to the phthalide constituents of celery seed (Oiye and Muroki 2002; Raffa 2005; Riddle 2004) although there may be other chemical components responsible for these effects. Among these are the active constituents of celery seed present in the 1.5–3% volatile oils which comprise monoterpene hydrocarbons (46%) and phthalides (42.3%) (Bjeldanes and Kim 1977; MacLeod et al. 1989). The ethanolic extracts of celery seeds (A-CSE) have been found to produce anti-inflammatory and anti-ulcer activity in rodent models (Whitehouse et al. 2000, 2001; Kitajima et al. 2002; Butters et al. 1999; Michio 2005). These extracts have also been found to variously affect prostaglandin production (K. D. Rainsford, 2001, unpublished observations). Aqueous extracts of celery stem have been found to have anti-inflammatory in the rat carrageenan foot paw edema model and in mouse ear histamine vascular inflammation (Lewis et al. 1985). Essential oil components of celery seed have been shown to have antioxidant activity in vitro (Wei and Shibamoto 2007). An aqueous/ethanolic extract of celery leaves and a purified component from this, *Apiin*, inhibits inducible nitric oxide (iNOS, NOS-II) and nitric oxide (NO) production in vitro as well as the croton oil-induced ear inflammation in mice (Mencherini et al. 2007). Phthalides of celery seed oil have been shown to inhibit benzo[α]pyrene-induced forestomach cancer in mice (Zheng et al. 1993).

Friedmen (2002) showed that celery seed extracts have bactericidal activities against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes* and *Salmonella enterica* (Friedman 2002), while a methanolic extract has antifungal, mosquitocidal and nematicidal activities (Momin et al. 2000; Momin and Nair 2001). Recently, Zhou et al. (2009) showed that the alcoholic extract of celery seed (A-CSE) and a phthalide dimer isolated from this extract inhibited *Helicobacter pylori* in vitro (Rainsford and Liu 2006).

These pharmacological effects suggest that there may be potential therapeutic applications for A-CSE in the treatment of a wide range of inflammatory and infectious conditions. In view of this, it was decided to undertake toxicological investigations to determine the safety of standardized A-CSE. Accordingly, a 28-day repeated oral dose study of A-CSE was undertaken in Sprague-Dawley rats. A-CSE is the starting material for all further purification or fractionation procedures. A-CSE is expressed in seed equivalent rather than absolute terms, for example, A-CSE 5,000 mg is an amount of extract equivalent to 5 g of green seed, freshly harvested and not dried, of Indian sourced material. Each 1,000 mg of seed yields approximately 76 mg of active extract.

Materials and methods

The alcoholic extract of celery seed (A-CSE) was a gift from Beagle International Pty Ltd (Nerang, QLD, Australia). This is a standardized preparation which has a reproducible HPLC fingerprint, details of which will be published elsewhere (Rainsford et al. 2010, in preparation).

The toxicological investigations were conducted by The Procter & Gamble Non-Clinical Testing Laboratory, Cincinnati, OH, USA in accordance with the United States Food and Drug Administration' Good Laboratory Practice (GLP) Regulations Title 21 of the US code of Federal Regulations Part 58, with the following exceptions:

1. The alpha-2-μ-globulin immunohistochemical staining was conducted using systems that were not GLP compliant.

2. Analysis of cytochrome P450 was conducted using systems that were not GLP compliant.

Rat studies

Eighteen adult male and 18 adult female rats were randomly assigned to 3 treatment groups of 6 rats/sex/group and were administered doses of A-CSE of 0, 150 or 5,000 mg/kg per day. Methylcellulose (0.5%) was used as the control and to dilute the A-CSE. Food was provided ad libitum during the study except the night prior to necropsy. Water was provided ad libitum during the study. The animals were observed twice daily (a.m. and p.m.) for morbidity and moribundity. Body weights, food consumption, and detailed clinical observations were recorded prior to initiation of treatment and at least weekly thereafter. During each detailed clinical observation interval, performed in conjunction with body weight measurements, each animal was assessed and findings recorded. Ophthalmology examinations were performed prior to study initiation and during week 4. After 28 days of treatment, all study animals were fasted overnight and urine was collected for 16–18 h. Just prior to necropsy, animals were anaesthetized with carbon dioxide and blood samples were collected via the vena cava for haematology, clinical chemistry and coagulation evaluation ("Appendix"). At necropsy, terminal body weights and macroscopic observations were recorded and organ weights and tissues were collected ("Appendix"). Histopathology was performed on all tissues from the vehicle control and the high dose (5,000 mg/kg per day) animals. In addition, the kidney tissues of the low dose animals were also processed and evaluated by light microscopy. Alpha-2-μ-globulin immunohistochemistry was performed on kidney tissues from all animals. Total CYP450, total form-specific activity and total microsomal protein were determined on liver tissue from all animals.

Microsomal cytochrome P-450 studies

Hepatic microsomes were prepared from 12 rats from each group (6 male, 6 female) of animals treated as above. The protein content of the microsomes (Bradford 1976) and enzymic activity of the cytochrome P-450 isozymes (CYP1A1/2, CYP2B1/2, CYP3A1/2,) were determined (Burke et al. 1985; Sonderfan et al. 1987).

Results and discussion

Table 1 depicts the changes in body weight from day 0 to day 28. That data from day 0 indicate that randomization by weight was achieved. There are no significant differences in weight gains in either male or female CSE treated animals compared with their respective controls whether measured as final weights at 28 days or absolute weight gains. Weight loss would be expected if there was a significant systemic toxic effect of the extract.

Statistically significant increased liver weight and liver to body and brain weight ratios in high dose females and liver to body weight ratios in high dose males were considered a test article effect (Table 2), but the absence of microscopic correlates for the liver weight increases suggests that these increases were not toxicologically significant.

Treatment related macroscopic changes were not observed at necropsy and microscopic findings were limited to minimal increases in gastric eosinophils in several male and female rats in the 5,000 mg/kg per day treatment groups. Minimal focal degeneration of renal tubules was observed sporadically in both sexes assigned to all treatment groups including control and was consistent with early spontaneous nephropathy of laboratory rats and thus was not considered to represent a pathologic change associated with the test article.

Minor corneal mineralization occurred in some animals from all treatment groups. Cataracts were observed in one in the control and one in an animal that had 5,000 mg/kg per day but since the cataracts occurred in the metabolically inactive region of the lens, these were not considered indicative of test article related lesions. There were no other remarkable findings at necropsy or in histopathology.

Since there were no statistically significant changes in haematology or measures of coagulation and only a few significant differences in clinical chemistry, only those variables which displayed significant changes are shown in Table 3. Male rats in the 5,000 mg/kg per day group had a statistically significant increase in serum globulin concentration and an increase in total serum protein (data not shown) consistent with the observed increase in serum globulin. The increase in serum phosphorus in male rats appears not to correlate with any other chemical measure or histological observation. Female rats exhibited significantly decreased levels of serum triglycerides and both the 150 and 5,000 mg/kg per day doses. Male rats also exhibited a decrease in mean serum triglycerides values of similar magnitude in both treated groups, but due to the large standard deviations these were not statistically significant (data not shown). No effect on cholesterol was seen in either sex. A recent report confirmed the decrease in triglycerides in male rats, but also noted a decrease in cholesterol after a 60-day exposure (Kamal et al. 2009).

Cytochrome P450 is a family of isozymes responsible for the biotransformation of many drugs (Amacher 2010).

Table 1 Body weight group summary

Variable	Statistics	0 mg/kg per day	150 mg/kg per day	5,000 mg/kg per day
Day 0	Mean	266.12	266.83	268.68
Males (g)	SD	15.89	12.02	13.80
	N	6	6	6
	Prob.		0.994	0.929
Day 28	Mean	423.02	418.90	412.10
Males (g)	SD	34.79	30.27	25.17
	N	6	6	6
	Prob.		0.960	0.762
Day 28	Mean	150.22	144.05	136.05
Males	SD	19.85	18.54	18.28
Absolute wgt gain (g)	*N*	6	6	6
	Prob.		0.798	0.350
Day 0	Mean	195.48	194.30	195.10
Females (g)	SD	11.73	11.81	10.66
	N	6	6	6
	Prob.		0.976	0.998
Day 28	Mean	247.98	251.13	247.48
Females (g)	SD	18.23	13.86	19.89
	N	6	6	6
	Prob.		0.944	0.998
Day 28	Mean	48.12	47.88	51.87
Females	SD	9.47	14.78	10.42
Absolute wgt gain (g)	*N*	6	5	6
	Prob.		0.999	0.802

Dunnett *P* values are shown to compare the control group (0 mg/kg per day) to the treated groups

Table 2 Organ weights and organ weight ratios day 28

Variable	Statistics	0 mg/kg per day	150 mg/kg per day	5,000 mg/kg per day
Females	Mean	7.255	7.658	8.401*
Liver (g)	SD	0.721	0.637	0.615
	N	6	5	6
	Prob.		0.517	0.017
Females	Mean	32.005	33.664	36.316**
Liver/body (g/kg)	SD	1.671	2.8772	1.730
	N	6	5	6
	Prob.		0.352	0.006
Females	Mean	3.917	4.251	4.614**
Liver/brain (g/g)	SD	0.296	0.385	0.382
	N	6	5	6
	Prob.		0.240	0.008
Males	Mean	33.552	33.350	36.250*
Liver/body (g/kg)	SD	1.648	1.288	2.387
	N	6	6	6
	Prob.		0.974	0.040

Dunnett *P* values are shown to compare the control group (0 mg/kg per day) to the treated groups

* $P < 0.05$; ** $P < 0.01$

Drug metabolism via the cytochrome P450 system has emerged as an important determinant in the occurrence of several drug interactions that can result in drug toxicities, reduced pharmacological effect, and adverse drug reactions. Recognizing whether the drugs involved act as enzyme substrates, inducers, or inhibitors can prevent clinically significant interactions from occurring. Eight different human P450 isozymes, CYP-1A2, -2B6, -2C8, -2C9, -2C19, -2D6, -2E1, and -3A4,5,7, that play clinically relevant roles in drug metabolism have been identified. A

Table 3 Clinical chemistries day 28

Variable	Statistics	0 mg/kg per day	150 mg/kg per day	5,000 mg/kg per day
Males	Mean	2.97	2.97	3.27*
Globulin (g/dL)	SD	0.12	0.18	0.24
	N	6	6	6
	Prob.		1.000	0.025
Males	Mean	11.37	11.27	12.50*
Phosphorus (mg/dL)	SD	0.83	0.70	0.74
	N	6	6	6
	Prob.		0.963	0.038
Females	Mean	23.2	15.3*	15.7*
Triglycerides (mg/dL)	SD	5.0	3.6	5.5
	N	6	6	6
	Prob.		0.022	0.028

Dunnett *P* values are shown to compare the control group (0 mg/kg per day) to the treated groups

* $P < 0.05$

Table 4 Total rat hepatic microsomal protein day 28

Treatment group	Average mg protein	Range of total protein mg
Control female	15.50	11.1–19.6
Control male	23.20	13.0–32.6
150 mg/kg Female	18.28	11.7–25.1
150 mg/kg Male	22.17	8.5–32.4
5,000 mg/kg Female	18.42	13.0–22.5
5,000 mg/kg Male	24.47	18.8–28.3

Table 5 Total rat hepatic CYP450 protein day 28

Treatment group	Average nmol P450/mg protein	Range of total CYP450 nmol
Control female	0.50	0.43–0.54
Control male	0.44	0.27–0.61
150 mg/kg Female	0.40	0.32–0.48
150 mg/kg Male	0.58	0.43–0.71
5,000 mg/kg Female	0.49	0.37–065
5,000 mg/kg Male	0.52	0.43–0.64

comprehensive listing of P450 substrates, inducers and inhibitors has been compiled by Flockhart (2009).

Hepatic microsomes prepared from male rats treated with the A-CSE preparations appeared to have higher levels of total microsomal protein in comparison to those prepared from the treated females, but there did not appear to be any effect of treatment (Table 4).

As shown in Table 5 the total cytochrome P450 did not appear to be different between groups nor affected by treatment.

As regards the activity of the various isoforms tested (CYP2B1/2, CYP1A1/2, CYP3A1/2), with the large range of activities detected for each P450 isoform, no clear change in activity or protein were observed, however, these data were not statistically analyzed.

There are many other extracts of celery seed on the market and biological activity varies greatly from one preparation to another, some preparations having little or no anti-inflammatory activity (Butters and Whitehouse 2003). It is thus likely that the toxicity of these preparations will also vary. Thus, the conclusions in this paper only refer to an alcoholic extract of green Indian celery seed as supplied by Beagle International Pty Ltd (Nerang, QLD, Australia).

Under the conditions for this study, the no adverse effect level for systemic toxicity was considered to be 5,000 mg/kg per day. Using a generally accepted formula for converting the animal dose in mg/kg to the human equivalent dose in mg/kg by correcting for interspecies differences in body surface area (CDER 2005), the 5,000 mg/kg dose in rats equals 810 mg/kg in adult humans or 56.76 g/day for a 70 kg person.

In a chronic model of pain and inflammation in the rat, 1500 mg/kg per day was shown to be as effective as either naproxen (30 mg/kg) or ibuprofen (100 mg/kg) but with a lag time of about 4–5 days before a similar degree of efficacy was observed (Rainsford et al. 2010, in preparation). Using the same body surface area correction factor used above, this would translate into a human dose of 243 mg/kg or 17.03 g/day for a 70 kg person. However, in a small clinical trial of 15 patients, a dose of 1,360 mg per day provided a 45–50% decrease in pain (Paul Sweeney, unpublished observations). Thus, as regards safety versus efficacy in animals there is at least a threefold margin and in the case of humans, if the clinical trial is representative, then this would represent an approximate 40-fold margin.

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Appendix

See Tables 6 and 7.

Table 6 Haematology, clinical chemistry and coagulation variables

Haematology: whole blood samples were analyzed for the following parameters	
Total leukocyte count (WBC)	White blood cell differential
Erythrocyte count (RBC)	Reticulocyte count
Hemoglobin concentration (HGB)	Mean corpuscular volume
Platelet count (PLT)	Mean corpuscular hemoglobin
Blood smear evaluation (morphology)	Mean corpuscular hemoglobin conc.
Hematocrit (HTC)	
Clinical chemistry: serum samples were analyzed for the following parameters	
Glucose (GLUC)	Total cholesterol (CHOL)
Urea nitrogen (UN)	Triglycerides (TRIG)
Total protein (TPROT)	Creatinine (CREAT)
Albumin (ALB)	Globulin (GLOB)
Albumin/globulin ratio (A/G)	Total bilirubin (TBILI)
Sodium (NA)	Aspartate aminotransferase (AST)
Potassium (K)	Alkaline phosphatase (ALP)
Chloride (CL)	Gamma glutamyltransferase (GGT)
Calcium (CA)	Sorbitol dehydrogenase (SDH)
Phosphorus (P)	Alkaline phosphatase (ALT)
Total bile acids (TBA)	
Coagulation: plasma samples were analyzed for the following parameters	
Prothrombin time (PT)	Activated partial thromboplastin time (APTT)

Table 7 Tissues collected for necropsy

Adrenal gland (2)[a]	Pituitary gland[a]
Aorta	Prostate gland[a]
Brain (cerebrum, cerebellum, medulla)[a]	Salivary glands (mandibular, 2)
Epididymides (2)[a]	Sciatic nerve
Esophagus	Seminal vesicles (2)
Eyes (with optic nerve)	Skeletal muscle (thigh)
Femur with bone marrow	Skin
Harderian glands (2)	Spinal cord (cervical, thoracic, lumbar)
Heart[a]	Spleen[a]
Intestine, large cecum; colon (proximal and distal); rectum	Sternum with bone marrow
Intestine, small duodenum; ileum; jejunum	Stomach (non-glandular and glandular)
Joints (femoro-tibial)	Testes (2)[a]
Kidneys (2)[a]	Thymus[a]
Lacrimal glands (exorbital, 2)	Thyroid/parathyroid[a]
Liver[a]	Tongue
Lungs	Trachea
Lymph nodes: submandibular, mesenteric	Urinary bladder
Mammary glands	Uterus with horns and cervix[a]
Ovaries (2)[a]	Vagina
Pancreas	

Histopathology was performed on all tissues from the vehicle control and the high dose (5,000 mg/kg per day) animals

[a] The weights of the selected organs were recorded at necropsy

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