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Cycloastragenol, a Proven Safety Modern Dietary Ingredient *

ABSTRACT

Cycloastragenol (CAG), a bioactive triterpene aglycone from Astragalus root extracts, is being developed as a modern dietary ingredient. To this end, studies assessing subchronic toxicity and genotoxic potential were conducted. In the subchronic study with recovery component, rats ingested 0, 40, 80, or 150 mg/kg/d CAG by oral gavage for ÿ91 consecutive days. No treatment-related mortalities occurred and no cardiac effects were identified. Although several endpoints among those monitored (i.e., clinical observations, body weight, food consumption, ophthalmology, urinalysis, hematology, clinical chemistry, gross pathology, organ weights, or histopathology) exhibited statistically significant effects, none was adverse. The oral no-observed-adverse-effect level (NOAEL) for CAG was >150 mg/kg/d in male and female rats.

1. INTRODUCTION

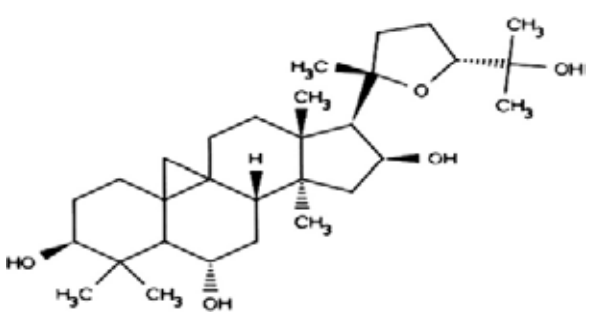

Fig. 1. Structure of cycloastragenol.

Currently available in US markets as a dietary supplement (Ganzera et al., 2001; Xiao et al., 2011), Radix Astragali is among the most popular of the Chinese herbs (Ma et al., 2002; Yu et al., 2007) where it is used alone and in combination with other ingredients. In traditional preparations, a decoction or soup made from the sliced or shredded root is taken to treat deficiencies in organs such as the spleen or lungs. Current preparations include teas and extracts readied from the ground or powdered root taken to enhance the immune system, increase stamina, and as a cardiotonic agent to strengthen or increase cardiac output (McKenna et al., 2002; WHO, 1999).

Cycloastragenol (CAG) is a secondary metabolite isolated from Radix Astragali. Present in all known Astragalus spp., CAG (Fig. 1) is both a triterpene aglycone and the most common genuine aglycone in the bioactive triterpenoid saponins called astragalosides (ASTs) (Rios and Waterman, 1997). Although CAG and the ASTs are found in all tissues of the Astragalus shrubs, the highest concentrations are localized in the roots (Yu et al., 2007b). Ten out of the eleven ASTs found in the root of A. membranaceus contain CAG as the aglycone, including AST IV, the primary characteristic and main bioactive AST present in the roots of this species (Kitagawa et al., 1983; McKenna et al., 2002; Sevimli-Gur et al., 2011; Verotta and El-Sebakhy, 2001; Yu et al., 2007a; Zhou et al., 2012). In the course of isolating, identifying,

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and testing the components of the major bioactive fractions (i.e., saponins, polysaccharides and isoflavones), cardiovascular effects such as hypotension induced by Astragalus extracts, have been attributed primarily to the activities of AST IV, a molecule which has been demonstrated to exert a positive inotropic effect (Verotta and El-Sebakhy, 2001; Li and Cao, 2002; Rios and Waterman, 1997; Yu et al., 2007a). CAG, AST IV, and other related molecules isolated from Astragalus spp. have also been identified as small-molecule telomerase activators, substances that can induce the elongation of telomeres, the protective DNA sequences at the terminal ends of chromosomes (de Jesus et al., 2011; Fauce et al., 2008; Harley et al., 2011; Yang et al., 2012; Yung et al., 2012; Zhou et al., 2012).

To support the development of CAG as a modern dietary ingredient for human consumption, the ingested safety of CAG was evaluated in the rat model via a 13-week repeated dose study with 4-week recovery period. Clinical observations, blood and urine assays, and gross and microscopic pathology were conducted to monitor for any sign of induced toxicity in the rat model. Although cardiotonic effects associated with Astragalus root extracts have not been attributed to CAG, a cardio-component consisting of blood pressure measurement and selected serum enzymes was also conducted. Reversibility, progression and/or the appearance of delayed changes were evaluated in additional sets of treatment and control animals assigned to the recovery study.

2. METHODS

In the main toxicity study, rats were assigned to groups (10/sex/group) that received 0 (Group 1, vehicle control), 40 (Group 2), 80 (Group 3) or 150 (Group 4) mg/kg bw/day CAG in aqueous-based vehicle via oral gavage (10 ml/kg bw/day) for 91 consecutive days. In the recovery study, additional groups (5/sex/group) received 0 (Group 5, vehicle control) or 150 (Group 6) mg/kg bw/day CAG for 91 consecutive days, then were observed post-treatment for 28 days. Surviving rats were terminated on Day 92 (male) or Day 93 (female) in the main toxicity study and on Day 120 in the recovery study. The stability, homogeneity and dose concentration of CAG in the test article and test formulations were confirmed using a validated high performance liquid chromatography method with evaporative light scattering detection.

Clinical signs were reported twice daily during study and recovery periods and on the morning of necropsy. Detailed observations were made on Day 1 before initial dosing and weekly thereafter. Body weights and food consumption were also recorded on Day 1, weekly during the dosing and recovery periods, and on the day of necropsy. During Week 12 at the end of the exposure period, a blind functional observational battery (FOB) was performed on all surviving animals in the main toxicity study. Ophthalmological examinations (both eyes) were conducted on all rats during the acclimation period and surviving main toxicity animals on Day 90. The cardiac assessment consisted of selected serum biochemistry parameters and tail-cuff blood pressure measurements. Blood for aspartate aminotransferase (AST), total creatine phosphokinase (CK), and lactate dehydrogenase (LDH) was collected on Day 86 (main toxicity) and Day 118 (recovery). After a two-week procedural acclimation, tail-cuff blood pressures were also collected (Week 12 for main study and Week 16 for recovery). Each animal was evaluated five times with each 30-s evaluation consisting of an average of five data points.

All animals were fasted ÿ15 h prior to sample collection for clinical pathology measures. Urine, collected on Day 86 (main study) and Day 118 (recovery study), was examined for quality, color, clarity, volume, microscopic urine sediments, pH, glucose, specific gravity, protein, ketone, bilirubin, blood, and urobilinogen. Blood for hematology and clinical chemistry (Tables 1 and 2) was collected via sublingual bleeding under isoflurane anesthesia on the same days. On Day 92 (males) or 93 (females), the main study rats, having been fasted for at least 15 h, were euthanized by exsanguination from the abdominal aorta under isoflurane anesthesia and necropsied; on Day 120 all rats in the recovery study were euthanized in the same manner. At termination blood for coagulation parameters (prothrombin time, activated partial thromboplastin time) was collected via the inferior vena cava under isoflurane anesthesia from all rats. Organs and tissues were also removed, weighed and examined macroscopically from all rats. Organs and tissues from main study animals (but not recovery animals) were examined microscopically.

3. RESULTS

No treatment-related mortalities occurred during main toxicity or recovery phases, nor were any treatment-related changes in physical condition or behavior observed. Further, no statistically significant changes in body weight were noted for any treatment group compared with the control group (Fig. 2). On Days 22–29, both mean daily body weight gain and mean food efficiency in Group 2 males were significantly decreased compared to Group 1 control males ($P < 0.01$ and $P < 0.05$, respectively). In all other male treatment groups mean daily body weight gains and food consumption were comparable to the control group. Because decreased food efficiency in Group 2 males was sporadic, not dose-dependent and not accompanied by decreases in body weight or food consumption, the findings were incidental. Among females, mean daily food consumption in Group 2 on Days 36–43 ($P < 0.01$) and Group 4 on Days 36–43 ($P < 0.01$), Days 50–57 ($P < 0.05$) and overall (Days 1–91) ($P < 0.05$) was significantly increased compared to Group 1 controls. Because the increased food consumption observed in Group 2 and 4 females was sporadic, not dose-dependent, and not accompanied by corresponding changes in body weight gain or food efficiency, the findings were incidental. No ophthalmological abnormalities were identified in any animals prior to study initiation or on Day 90. No statistically significant effects were reported for urinalysis parameters (data not shown) in any surviving rats on Day 86 (main toxicity) or Day 118 (recovery).

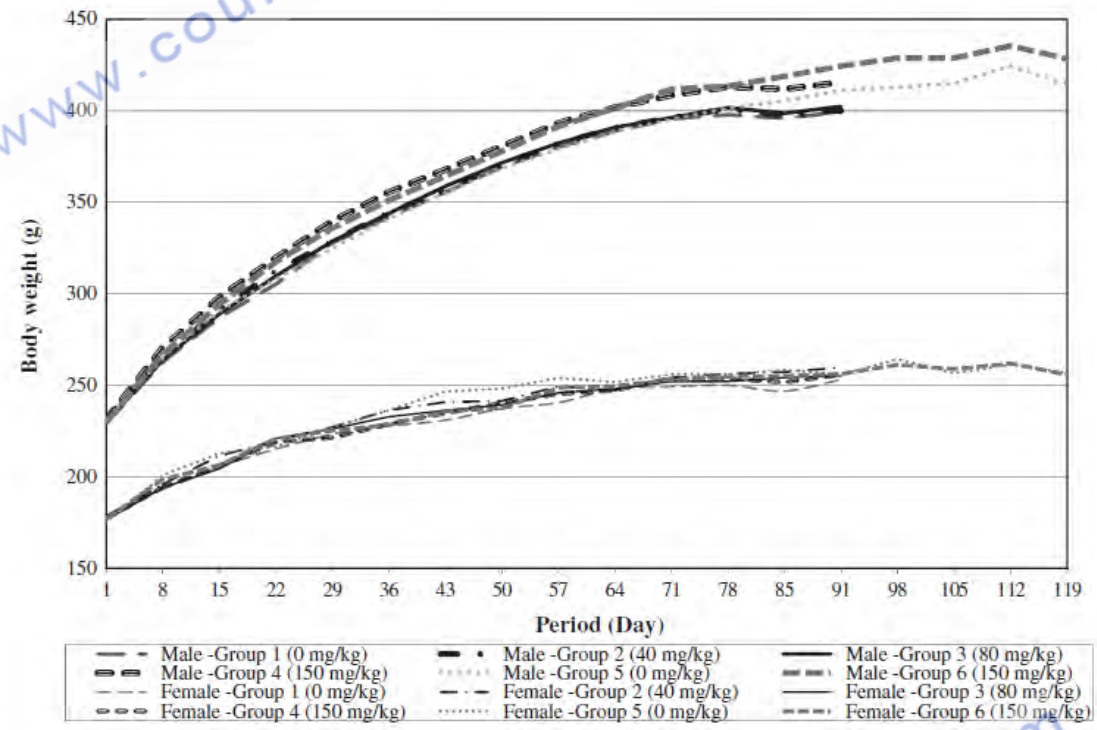

Fig. 2. Mean body weights of male and female rats during 13-week treatment and 4-week recovery periods.

Clinical chemistry results (Tables 1 and 2) on Day 86 revealed statistically significant decreases in alkaline phosphatase and total cholesterol in Group 3 males, triglycerides in Group 2 and Group 3 males, and bilirubin in Group 2, 3 and 4 males, when compared to Group 1 control males ($P < 0.05$ for all). On Day 118 in Group 6 females, statistically significant decreases were noted in creatinine and cholesterol, along with statistically significant increases in calcium, potassium, and inorganic phosphorus concentrations compared to Group 5 control females ($P < 0.05$ for all). None of these findings was judged to be toxicologically significant, as none demonstrated a dose-dependent relationship, was accompanied by any other clinical or histopathologic changes, and/or was outside the historical range observed in the age and strain of rat used (Pettersen et al., 1996; Derelanko, 2000).None of the coagulation parameters in the main toxicity (Day 92/93) or recovery (Day 120) studies was affected by treatment (Table 1 and 2).

pressure values) (data not shown) was identified between the dose groups and the corresponding control groups for either sex in the main toxicity or recovery study. In addition, the Week 16 blood pressure measures for Groups 5 and 6 and Week 12 values for Groups 1 and 4 were comparable. No statistically significant changes in the serum biochemistry parameters, AST, CK or LDH were identified in male or female rats in the main toxicity (Day 86) or recovery (Day 118) studies (Tables 1 and 2).

Table 1
Hematology, coagulation, and clinical chemistry parameters in male rats following 86 or 91 days of treatment with CAG[a].

Parameter	Main toxicity study				Recovery study	
	Group 1 0 mg/kg bw/d	Group 2 40 mg/kg bw/d	Group 3 80 mg/kg bw/d	Group 4 150 mg/kg bw/d	Group 5 0 mg/kg bw/d	Group 6 150 mg/kg bw/d
Hematology – Day 86 Main Toxicity/Day 118 Recovery						
RBC (10^6/μl)	9.16 ± 0.47[b]	8.86 ± 0.42	9.06 ± 0.25	8.91 ± 0.30	9.48 ± 0.35[c]	9.25 ± 0.36
Hemoglobin (g/dl)	15.6 ± 0.8[b]	15.4 ± 0.6	15.8 ± 0.5	15.6 ± 0.7	16.2 ± 0.3[c]	16.1 ± 0.7
Hematocrit (%)	46.7 ± 2.6[b]	46.1 ± 1.8	47.2 ± 1.6	45.9 ± 2.0	49.1 ± 1.3[c]	48.8 ± 2.6
MCV (fl)	51.0 ± 1.0[b]	52.1 ± 1.3	52.1 ± 0.7	51.5 ± 1.2	51.8 ± 2.0[c]	52.7 ± 1.8
MCH (pg)	17.1 ± 0.4[b]	17.4 ± 0.4	17.4 ± 0.3	17.5 ± 0.4*	17.0 ± 0.6[c]	17.5 ± 0.3
MCHC (g/dl)	33.5 ± 0.4[b]	33.5 ± 0.5	33.5 ± 0.6	34.0 ± 0.4	32.9 ± 0.2[c]	33.1 ± 0.5
RDW (%)	12.3 ± 0.4[b]	12.6 ± 1.0	12.1 ± 0.4	12.2 ± 0.3	12.2 ± 0.2[c]	12.2 ± 0.5
Platelet count (10^3/μl)	743 ± 148[b]	788 ± 128	800 ± 156	754 ± 79	855 ± 61[c]	922 ± 72
WBC (10^3/μl)	11.28 ± 1.32[b]	10.92 ± 1.34	10.68 ± 1.47	11.22 ± 2.38	11.41 ± 2.77[c]	11.01 ± 0.58
ARET (10^3/μl)	171.2 ± 24.4[b]	181.5 ± 75.8	164.4 ± 17.3	162.8 ± 22.1	194.6 ± 38.3	192.6 ± 10.0
ANEU (10^3/μl)	1.74 ± 0.49[b]	1.61 ± 0.57	1.42 ± 0.49	1.55 ± 0.62	1.55 ± 0.78[c]	1.40 ± 0.16
ALYM (10^3/μl)	8.87 ± 0.95[b]	8.66 ± 1.07	8.64 ± 1.08	8.99 ± 2.18	9.28 ± 2.18[c]	9.00 ± 0.51
AMON (10^3/μl)	0.31 ± 0.08[b]	0.32 ± 0.09	0.30 ± 0.06	0.35 ± 0.11	0.26 ± 0.07[c]	0.30 ± 0.09
AEOS (10^3/μl)	0.16 ± 0.05[b]	0.16 ± 0.05	0.17 ± 0.04	0.17 ± 0.04	0.14 ± 0.02[c]	0.14 ± 0.04
ABAS (10^3/μl)	0.09 ± 0.03[b]	0.08 ± 0.03	0.07 ± 0.03	0.08 ± 0.04	0.10 ± 0.05[c]	0.08 ± 0.03
ALUC (10^3/μl)	0.10 ± 0.03[b]	0.09 ± 0.03	0.09 ± 0.03	0.10 ± 0.04	0.09 ± 0.02[c]	0.09 ± 0.04
AATL (10^3/μl)	–	–	–	–	–	–
AIL (10^3/μl)	–	–	–	–	–	–
Coagulation – Day 92 Main Toxicity/Day 120 Recovery						
PT (s)	10.5 ± 0.3[b]	10.6 ± 0.2[b]	10.7 ± 0.2	10.6 ± 0.2[b]	10.3 ± 0.2	10.3 ± 0.2[c]
APTT (s)	17.7 ± 1.2[b]	17.7 ± 2.2[b]	18.6 ± 1.6	18.4 ± 0.9[b]	17.5 ± 0.6	17.0 ± 2.5[c]
Clinical Chemistry – Day 86 Main Toxicity/Day 118 Recovery						
AST (U/l)	79 ± 10[b]	78 ± 16	72 ± 10[b]	73 ± 7	99 ± 19	104 ± 25
ALT (U/l)	46 ± 7[d]	44 ± 5[b]	41 ± 4	40 ± 9	43 ± 5	44 ± 15
SDH (U/l)	6.0 ± 2.2[e]	8.0 ± 0.8[b]	7.1 ± 2.1	7.0 ± 1.4[d]	6.1 ± 2.1[c]	5.7 ± 0.9[e]
ALKP (U/l)	98 ± 18[b]	89 ± 17	79 ± 11*	85 ± 11	79 ± 26	64 ± 18
BILI (mg/dl)	0.12 ± 0.02[b]	0.10 ± 0.01*	0.10 ± 0.0*	0.10 ± 0.02[b],*	0.14 ± 0.02	0.15 ± 0.03
BUN (mg/dl)	18 ± 2[b]	18 ± 2	17 ± 2	17 ± 2	17 ± 0	17 ± 1
Creatinine (mg/dl)	0.31 ± 0.03[b]	0.30 ± 0.03	0.31 ± 0.03	0.29 ± 0.03	0.35 ± 0.03	0.36 ± 0.03
Total cholesterol (mg/dl)	104 ± 10[b]	98 ± 10	89 ± 9*	93 ± 14	74 ± 14	78 ± 19
Triglycerides (mg/dl)	56 ± 10[b]	43 ± 10*	40 ± 10*	45 ± 10	53 ± 11	55 ± 6
Glucose, fasting (mg/dl)	148 ± 27[b]	141 ± 20	151 ± 24	146 ± 24[b]	132 ± 17	138 ± 20
Total protein (g/dl)	6.1 ± 0.2[d]	5.9 ± 0.2*	6.1 ± 0.2	6.0 ± 0.3[b]	6.0 ± 0.1[c]	6.0 ± 0.2[e]
Albumin (g/dl)	3.1 ± 0.1[b]	3.2 ± 0.1	3.2 ± 0.1	3.1 ± 0.1	3.3 ± 0.1	3.2 ± 0.3
Globulin (g/dl)	3.0 ± 0.2[d]	2.8 ± 0.2[b]	3.0 ± 0.2	3.0 ± 0.3[b]	2.7 ± 0.2[c]	2.6 ± 0.1*
Calcium (mg/dl)	9.8 ± 0.3[b]	9.7 ± 0.5	10.0 ± 0.3	9.8 ± 0.6[b]	9.1 ± 0.4[c]	9.2 ± 0.2*
Inorganic phosphorus (mg/dl)	6.5 ± 0.4[b]	6.5 ± 0.4	6.3 ± 0.5	6.8 ± 0.5	6.2 ± 0.2	6.1 ± 0.3
Sodium (mmol/l)	142.1 ± 1.3[b]	142.8 ± 2.1	142.2 ± 1.2	142.3 ± 1.5	140.2 ± 1.3	140.1 ± 1.9
Potassium (mmol/l)	5.59 ± 0.36[b]	5.54 ± 0.20	5.59 ± 0.31	5.66 ± 0.39	4.91 ± 0.13	4.92 ± 0.12
Chloride (mmol/l)	103.7 ± 1.2[b]	103.9 ± 1.3	103.5 ± 1.2	103.1 ± 1.2	101.4 ± 1.3	101.0 ± 1.8
CK (U/l)	207 ± 96[b]	176 ± 71	187 ± 65	176 ± 44	151 ± 72	153 ± 57
LDH (mmol/l)	610 ± 312[b]	539 ± 263	529 ± 205	549 ± 120[b]	339 ± 118	363 ± 176

* $P < 0.05$, compared to control.
[a] All data are presented as mean values ± standard deviations with $N = 10$ for main toxicity groups and $N = 5$ for recovery groups, except when otherwise indicated.
[b] $N = 9$.
[c] $N = 4$.
[d] $N = 8$.
[e] $N = 7$.

Table 2
Hematology, coagulation, and clinical chemistry parameters in female rats following 86 or 92 days of treatment with CAG[a].

Parameter	Main toxicity study				Recovery study	
	Group 1 0 mg/kg bw/d	Group 2 40 mg/kg bw/d	Group 3 80 mg/kg bw/d	Group 4 150 mg/kg bw/d	Group 5 0 mg/kg bw/d	Group 6 150 mg/kg bw/d
Hematology – Day 86 Main Toxicity/Day 118 Recovery						
RBC (10^6/µl)	8.04 ± 0.26	8.22 ± 0.25	7.97 ± 0.10	8.23 ± 0.20	8.37 ± 019	8.53 ± 0.29
Hemoglobin (g/dl)	14.7 ± 0.6	14.8 ± 0.5	14.7 ± 0.4	14.8 ± 0.2	15.1 ± 0.4	15.0 ± 0.6
Hematocrit (%)	42.8 ± 2.1	43.5 ± 1.2	43.0 ± 0.7	43.7 ± 1.2	45.0 ± 0.9	45.0 ± 1.4
MCV (fl)	53.3 ± 1.4	53.0 ± 1.4	54.0 ± 1.0	53.1 ± 1.1	53.7 ± 0.8	52.8 ± 1.8
MCH (pg)	18.2 ± 0.6	18.0 ± 0.5	18.5 ± 0.5	18.0 ± 0.3	18.1 ± 0.4	17.6 ± 0.7
MCHC (g/dl)	34.3 ± 0.9	33.9 ± 0.6	34.3 ± 0.8	33.9 ± 0.5	33.7 ± 0.2	33.3 ± 0.5
RDW (%)	11.2 ± 0.3	11.3 ± 0.3	11.3 ± 0.3	11.3 ± 0.3	10.8 ± 0.3	11.2 ± 0.5
Platelet count (10^3/µl)	807 ± 137	837 ± 133	775 ± 115	880 ± 212	954 ± 208	947 ± 68
WBC (10^3/µl)	6.74 ± 1.48	7.07 ± 2.18	6.79 ± 1.32	7.21 ± 1.41	6.09 ± 0.30	7.79 ± 1.22*
ARET (10^3/µl)	138.8 ± 22.5	170.3 ± 32.9	182.8 ± 30.1*	169.1 ± 40.4	166.2 ± 20.4	170.6 ± 53.2
ANEU (10^3/µl)	0.75 ± 0.24	1.02 ± 0.55	0.85 ± 0.29	1.02 ± 0.38	0.67 ± 0.15	0.86 ± 0.36
ALYM (10^3/µl)	5.62 ± 1.43	5.66 ± 1.62	5.54 ± 1.16	5.79 ± 1.29	5.09 ± 0.16	6.54 ± 0.93*
AMON (10^3/µl)	0.15 ± 0.04	0.16 ± 0.07	0.16 ± 0.03	0.16 ± 0.05	0.14 ± 0.01	0.16 0.06
AEOS (10^3/µl)	0.12 ± 0.05	0.15 ± 0.06	0.15 ± 0.05	0.16 ± 0.06	0.12 ± 0.02	0.12 ± 0.02
ABAS (10^3/µl)	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.02	0.03 ± 0.01	0.04 ± 0.02
ALUC (10^3/µl)	0.05 ± 0.04	0.05 ± 0.03	0.06 ± 0.01	0.06 ± 0.02	0.04 ± 0.01	0.07 ± 0.04
AATL (10^3/µl)	0.06[b]	–	–	–	–	–
AIL (10^3/µl)	0.12[b]	–	–	–	–	–
Coagulation – Day 93 Main Toxicity/Day 120 Recovery						
PT (sec)	10.3 ± 0.2	10.3 ± 0.2	10.3 ± 0.2	10.3 ± 0.4	9.9 ± 0.1	10.0 ± 0.1
APTT (s)	16.3 ± 1.2	16.7 ± 1.3	16.0 ± 1.2	16.2 ± 1.9	15.6 ± 1.6	15.2 ± 1.0
Clinical Chemistry – Day 86 Main Toxicity/Day 118 Recovery						
AST (U/l)	75 ± 14	77 ± 8	78 ± 7	73 ± 10	75 ± 7	68 ± 4
ALT (U/l)	33 ± 5	33 ± 4	35 ± 3	33 ± 4	37 ± 3	35 ± 3
SDH (U/l)	6.0 ± 1.9[c]	5.3 ± 1.2[c]	5.1 ± 1.4	6.4 ± 1.1	3.5 ± 1.2[d]	3.5 ± 2.6[d]
ALKP (U/l)	69 ± 12	71 ± 22	64 ± 12	71 ± 14	71 ± 14	66 ± 14
BILI (mg/dl)	0.14 ± 0.02	0.14 ± 0.01	0.16 ± 0.02	0.13 ± 0.02	0.16 ± 0.01[e]	0.15 ± 0.02
BUN (mg/dl)	21 ± 3	20 ± 3	19 ± 3	19 ± 2	20 ± 1	18 ± 2
Creatinine (mg/dl)	0.41 ± 0.02	0.42 ± 0.05	0.39 ± 0.04	0.40 ± 0.03	0.45 ± 0.03	0.37 ± 0.06*
Total cholesterol (mg/dl)	113 ± 23	103 ± 21	105 ± 16	115 ± 22	118 ± 12	89 ± 23*
Triglycerides (mg/dl)	34 ± 9	36 ± 7	31 ± 6	35 ± 5	35 ± 7	33 ± 5
Glucose, fasting (mg/dl)	120 ± 32	106 ± 12[c]	114 ± 14	102 ± 6	109 ± 11[d]	113 ± 8
Total protein (g/dl)	5.8 ± 0.3	5.8 ± 0.3[c]	5.9 ± 0.4	6.0 ± 0.3	5.5 ± 0.4[d]	5.7 ± 0.2[d]
Albumin (g/dl)	3.2 ± 0.2	3.2 ± 0.2	3.3 ± 0.2	3.3 ± 0.1	3.2 ± 0.3	3.1 ± 0.1
Globulin (g/dl)	2.6 ± 0.2	2.6 ± 0.2[c]	2.6 ± 0.2	2.7 ± 0.2	2.4 ± 0.1[d]	2.5 ± 0.2[d]
Calcium (mg/dl)	9.8 ± 0.2	9.6 ± 0.3[c]	9.7 ± 0.4	9.6 ± 0.4	8.5 ± 0.2[d]	8.9 ± 0.1[d,*]
Inorganic phosphorus (mg/dl)	5.5 ± 0.7	5.3 ± 0.7	5.5 ± 0.4	5.4 ± 0.4	4.7 ± 0.1	5.4 ± 0.5*
Sodium (mmol/l)	136.1 ± 2.1	135.5 ± 2.5[c]	135.9 ± 1.9	130.9 ± 10.6	141.3 ± 0.8	140.7 ± 0.8
Potassium (mmol/l)	4.89 ± 0.40	4.76 ± 0.35[c]	4.93 ± 0.33	4.48 ± 0.48	4.39 ± 0.14	4.74 ± 0.25*
Chloride (mmol/l)	100.6 ± 1.8	99.5 ± 2.2[c]	99.6 ± 1.6	96.2 ± 8.2	103.9 ± 1.1	103.5 ± 1.1
CK (U/l)	182 ± 105	190 ± 68	204 ± 49	152 ± 57	171 ± 87	128 ± 44
LDH (mmol/l)	494 ± 293	547 ± 207	591 ± 177	425 ± 210	425 ± 312	265 ± 159

* $P < 0.05$, compared to control.
[a] All data are presented as mean values ± standard deviations with $N = 10$ for main toxicity groups and $N = 5$ for recovery groups, except when otherwise indicated.
[b] $N = 1$.
[c] $N = 9$.
[d] $N = 3$.
[e] $N = 4$.

Analysis of absolute and relative organ weights revealed one statistically significant change in each of two male treatment groups when compared with their respective controls at termination (Table 3), increased absolute liver weight in Group 4 males ($P < 0.05$) and increased heart-to-brain weight ratio in Group 6 males ($P < 0.05$). Neither finding was not dose-dependent or had any clinical or histopathologic correlate; the observations were therefore determined to be incidental and not related to treatment. In Group 3 and Group 4 females (Table 4), statistically significant changes included increased absolute heart weight ($P < 0.05$ for both groups), heart-to-body weight ($P < 0.01$, Group 3; $P < 0.05$, Group 4) and heart-to-brain weight ratios ($P < 0.01$, Group 3; $P < 0.05$, Group 4). Although there appeared to be a dose-dependent trend across the control, low-dose, and intermediate-dose groups for these parameters (Table 4), only the intermediate-dose group differed from the control to a statistically significant degree. In addition, these same parameters in high-dose Group 4 – although significantly increased over the control – did not continue the trend; the values fell between those of the low- and intermediate-dose groups (Table 4).The observed changes, which did not correlate to any clinical or histopathologic findings, were not considered to be toxicologically relevant. The changes could potentially be considered adaptive and fully reversible as borne out by the recovery phase; no absolute or relative organ weights for Group 6 females in the recovery study, including those related to the heart, showed any effect.

Table 3
Terminal body weights and absolute and relative organ weights of male rats following 91 days of treatment with CAG[a].

Parameter	Main toxicity study – Day 92				Recovery study – Day 120	
	Group 1[b] 0 mg/kg bw/d	Group 2 40 mg/kg bw/d	Group 3 80 mg/kg bw/d	Group 4 150 mg/kg bw/d	Group 5 0 mg/kg bw/d	Group 6 150 mg/kg bw/d
Absolute weights						
Terminal body weight (g)	377.3 ± 16.1	380.8 ± 25.7	382.6 ± 18.8	395.4 ± 30.1	393.4 ± 21.1	407.0 ± 28.1
Adrenals (g)	0.0633 ± 0.0064	0.0643 ± 0.0099	0.0655 ± 0.0064	0.0598 ± 0.0073	0.0564 ± 0.0068	0.0560 ± 0.0058
Brain (g)	1.971 ± 0.063	1.963 ± 0.097	2.032 ± 0.059	2.011 ± 0.077	1.940 ± 0.097	1.906 ± 0.078
Epididymides (g)	1.368 ± 0.167	1.437 ± 0.093	1.418 ± 0.064	1.417 ± 0.110	1.428 ± 0.124	1.440 ± 0.122
Heart (g)	1.280 ± 0.099	1.234 ± 0.085	1.269 ± 0.114	1.280 ± 0.102	1.202 ± 0.083	1.320 ± 0.131
Kidneys (g)	2.581 ± 0.172	2.619 ± 0.205	2.600 ± 0.162	2.670 ± 0.195	2.454 ± 0.244	2.566 ± 0.301
Liver (g)	9.867 ± 0.498	9.859 ± 0.925	10.393 ± 0.820	10.918 ± 1.130*	10.060 ± 1.254	10.416 ± 1.054
Spleen (g)	0.773 ± 0.041	0.745 ± 0.104	0.771 ± 0.074	0.811 ± 0.085	0.766 ± 0.090	0.700 ± 0.035
Testes (g)	3.614 ± 0.568	3.724 ± 0.309	3.839 ± 0.218	3.959 ± 0.225	3.836 ± 0.379	4.074 ± 0.336
Thymus (mg)	0.2528 ± 0.0491	0.2736 ± 0.0909	0.2710 ± 0.0628	0.2908 ± 0.0262	0.1806 ± 0.0382	0.2054 ± 0.0314
Organ-to-body weight ratios						
Adrenals/BW	0.1679 ± 0.0161	0.1695 ± 0.0282	0.1717 ± 0.0202	0.1511 ± 0.0122	0.1431 ± 0.0115	0.1378 ± 0.0147
Brain/BW	5.229 ± 0.196	5.172 ± 0.379	5.322 ± 0.297	5.116 ± 0.483	4.934 ± 0.167	4.692 ± 0.179
Epididymides/BW	3.6355 ± 0.5060	3.7869 ± 0.3316	3.7160 ± 0.2796	3.5876 ± 0.1849	3.6294 ± 0.2365	3.5571 ± 0.4427
Heart/BW	3.390 ± 0.185	3.245 ± 0.178	3.314 ± 0.200	3.246 ± 0.256	3.056 ± 0.144	3.244 ± 0.234
Kidneys/BW	6.841 ± 0.357	6.884 ± 0.431	6.799 ± 0.356	6.762 ± 0.336	6.226 ± 0.293	6.290 ± 0.377
Liver/BW	26.162 ± 1.115	25.888 ± 1.645	27.142 ± 1.210	27.621 ± 2.031	25.506 ± 1.879	25.558 ± 1.220
Spleen/BW	2.055 ± 0.176	1.953 ± 0.198	2.014 ± 0.139	2.058 ± 0.225	1.955 ± 0.285	1.723 ± 0.088
Testes/BW	9.615 ± 1.701	9.790 ± 0.671	10.053 ± 0.730	10.064 ± 0.935	9.734 ± 0.478	10.020 ± 0.662
Thymus/BW	0.6707 ± 0.1321	0.7158 ± 0.2251	0.7087 ± 0.1659	0.7377 ± 0.0709	0.4601 ± 0.1010	0.5054 ± 0.0747
Organ-to-brain weight ratios						
Adrenals/BrW	0.0321 ± 0.0028	0.0329 ± 0.0056	0.0323 ± 0.0032	0.0298 ± 0.0041	0.0290 ± 0.0024	0.0294 ± 0.0030
Epididymides/BrW	0.6943 ± 0.0854	0.7344 ± 0.0688	0.6987 ± 0.0450	0.7059 ± 0.0641	0.7369 ± 0.0638	0.7565 ± 0.0713
Heart/BrW	0.650 ± 0.051	0.629 ± 0.037	0.626 ± 0.065	0.637 ± 0.053	0.620 ± 0.040	0.691 ± 0.044*
Kidneys/BrW	1.310 ± 0.083	1.334 ± 0.082	1.279 ± 0.069	1.330 ± 0.119	1.264 ± 0.092	1.343 ± 0.109
Liver/BrW	5.009 ± 0.268	5.029 ± 0.482	5.117 ± 0.409	5.442 ± 0.663	5.179 ± 0.485	5.460 ± 0.450
Spleen/BrW	0.393 ± 0.026	0.380 ± 0.057	0.380 ± 0.040	0.403 ± 0.040	0.395 ± 0.045	0.367 ± 0.019
Testes/BrW	1.835 ± 0.294	1.901 ± 0.178	1.891 ± 0.125	1.970 ± 0.121	1.977 ± 0.156	2.137 ± 0.151
Thymus/BrW	0.1280 ± 0.0226	0.1408 ± 0.0510	0.1332 ± 0.0299	0.1447 ± 0.0132	0.0931 ± 0.0189	0.1077 ± 0.0152

BW = body weight; BrW = brain weight; CAG = cycloastragenol.
* $P < 0.05$.
[a] All data are presented as mean values ± standard deviations with $N = 10$ for main toxicity groups and $N = 5$ for recovery groups, except when otherwise indicated;
[b] $N = 9$.

Table 4
Terminal body weights and absolute and relative organ weights of female rats following 92 days of treatment with CAG[a].

Parameter	Main toxicity study – Day 93				Recovery study – Day 120	
	Group 1[b] 0 mg/kg bw/d	Group 2 40 mg/kg bw/d	Group 3 80 mg/kg bw/d	Group 4 150 mg/kg bw/d	Group 5 0 mg/kg bw/d	Group 6 150 mg/kg bw/d
Absolute weights						
Terminal body weight (g)	240.5 ± 9.8	245.1 ± 8.9	241.7 ± 9.5	243.9 ± 15.6	243.0 ± 9.3	241.0 ± 6.4
Adrenals (g)	0.0744 ± 0.0088	0.0652 ± 0.0092	0.0698 ± 0.0101	0.0670 ± 0.0073	0.0644 ± 0.0079	0.0608 ± 0.0029
Brain (g)	1.902 ± 0.097	1.871 ± 0.081	1.838 ± 0.049	1.878 ± 0.062	1.828 ± 0.064	1.840 ± 0.050
Heart (g)	0.870 ± 0.051	0.916 ± 0.051	0.941 ± 0.032*	0.939 ± 0.079*	0.890 ± 0.047	0.920 ± 0.076
Kidneys (g)	1.658 ± 0.120	1.607 ± 0.064	1.665 ± 0.107	1.640 ± 0.112	1.566 ± 0.076	1.606 ± 0.106
Liver (g)	6.156 ± 0.396	6.131 ± 0.282	6.208 ± 0.496	6.454 ± 0.660	5.898 ± 0.308	5.858 ± 0.434
Ovaries (g)	0.0851 ± 0.0153	0.0968 ± 0.0314	0.0796 ± 0.0248	0.0925 ± 0.0175	0.0806 ± 0.0131	0.0970 ± 0.0393
Spleen (g)	0.643 ± 0.089	0.664 ± 0.087	0.624 ± 0.072	0.651 ± 0.081	0.618 ± 0.036	0.600 ± 0.070
Thymus (mg)	0.2114 ± 0.0461	0.2254 ± 0.0299	0.2281 ± 0.0368	0.2127 ± 0.0482	0.1338 ± 0.0408	0.1528 ± 0.0316
Uterus-Oviduct (g)	0.754 ± 0.237	0.681 ± 0.162	0.814 ± 0.274	0.558 ± 0.150	0.786 ± 0.288	0.808 ± 0.334
Organ-to-body weight ratios						
Adrenals/BW	0.3094 ± 0.0354	0.2668 ± 0.0430	0.2888 ± 0.0404	0.2750 ± 0.0275	0.2655 ± 0.0345	0.2525 ± 0.0162
Brain/BW	7.914 ± 0.379	7.642 ± 0.419	7.613 ± 0.301	7.724 ± 0.506	7.529 ± 0.325	7.639 ± 0.283
Heart/BW	3.621 ± 0.235	3.740 ± 0.213	3.896 ± 0.118**	3.849 ± 0.193*	3.668 ± 0.250	3.816 ± 0.270
Kidneys/BW	6.900 ± 0.496	6.561 ± 0.275	6.899 ± 0.529	6.728 ± 0.287	6.443 ± 0.130	6.669 ± 0.498
Liver/BW	25.590 ± 1.051	25.031 ± 1.212	25.705 ± 2.117	26.420 ± 1.424	24.265 ± 0.598	24.290 ± 1.329
Ovaries/BW	0.3543 ± 0.0638	0.3968 ± 0.1302	0.3294 ± 0.0989	0.3780 ± 0.0621	0.3305 ± 0.0417	0.4018 ± 0.1591
Spleen/BW	2.667 ± 0.279	2.714 ± 0.376	2.584 ± 0.293	2.668 ± 0.290	2.542 ± 0.078	2.487 ± 0.250
Thymus/BW	0.8771 ± 0.1803	0.9217 ± 0.1324	0.9433 ± 0.1448	0.8777 ± 0.2149	0.5521 ± 0.1753	0.6366 ± 0.1443
Uterus-Oviduct/BW	3.152 ± 1.038	2.775 ± 0.633	3.388 ± 1.210	2.302 ± 0.550	3.226 ± 1.152	3.367 ± 1.453
Organ-to-brain weight ratios						
Adrenals/BrW	0.0393 ± 0.0055	0.0349 ± 0.0052	0.0380 ± 0.0058	0.0357 ± 0.0043	0.0353 ± 0.0051	0.0330 ± 0.0009
Heart/BrW	0.458 ± 0.031	0.491 ± 0.038	0.512 ± 0.023**	0.500 ± 0.041*	0.487 ± 0.030	0.500 ± 0.035
Kidneys/BrW	0.872 ± 0.058	0.861 ± 0.062	0.907 ± 0.070	0.874 ± 0.057	0.857 ± 0.029	0.872 ± 0.040
Liver/BrW	3.238 ± 0.157	3.284 ± 0.223	3.380 ± 0.293	3.436 ± 0.314	3.228 ± 0.161	3.183 ± 0.201
Ovaries/BrW	0.0450 ± 0.0092	0.0517 ± 0.0165	0.0433 ± 0.0132	0.0493 ± 0.0094	0.0440 ± 0.0065	0.0527 ± 0.0211
Spleen/BrW	0.337 ± 0.035	0.355 ± 0.047	0.340 ± 0.046	0.348 ± 0.049	0.338 ± 0.017	0.326 ± 0.032
Thymus/BrW	0.1116 ± 0.0257	0.1204 ± 0.0138	0.1245 ± 0.0220	0.1134 ± 0.0261	0.0728 ± 0.0205	0.0833 ± 0.0183
Uterus-Oviduct/BrW	0.399 ± 0.131	0.364 ± 0.085	0.443 ± 0.151	0.298 ± 0.083	0.429 ± 0.159	0.438 ± 0.177

BW = body weight; BrW = brain weight; CAG = cycloastragenol.
* $P < 0.05$.
** $P < 0.01$.
[a] All data are presented as mean values ± standard deviations with $N = 10$ for main toxicity groups and $N = 5$ for recovery groups, except when otherwise indicated.
[b] $N = 9$.

Macroscopic findings in main toxicity males (Day 92) included small, bilateral testes, which histopathology later confirmed as due to slight germ cell atrophy, and a small, bilateral epididymis that was later determined to correspond to slight hypospermia in one Group 1 control male; a soft mass in the left epididymis due to the presence of a sperm granuloma in one Group 2 male; and a discolored liver with a supernumerary lobe in one Group 4 male that correspondedto chronic liver infarction consistent with liver lobe torsion. Each of these findings was incidental and not treatment-related. No macroscopic observations were reported in recovery males (Day 120). Incidental macroscopic findings in females included fluid-filled uteri/oviducts consistent with variations in the estrous cycle; an endometrial cyst in one Group 1 female; and multiple mass-like lesions embedded in fat and connective tissue in close proximity to the thymus and lungs in one control female. Microscopic evaluation of the latter finding was not confirmed, but was toxicologically insignificant, as it occurred in a control animal. Microscopic findings in control and high dose animals of the main study consisted of only incidental findings (i.e., minimal chronic progressive neuropathy commonly observed in SD rats; trauma to the esophageal walls consistent with daily gavage procedures; a mesenchymal tumor in one kidney of one Group 4 female). No adverse macroscopic or microscopic observations related to the test substance were noted for the heart. Under the conditions of this study, the no-observed-adverse-effect level (NOAEL) for orally administered CAG in the rat was 150 mg/kg bw/day (equivalent to 10,500 mg/day in a 70-kg individual), the highest dose tested.

4. DISCUSSION

In the 13-week subchronic toxicity study daily ingestion of CAG at the highest dose administered provided 150 mg/kg bw/d. This exposure was well-tolerated by the rats with no resulting toxicities or other adverse effects. Although the highest dose studied in rats is equivalent to 10,500 mg/d in a 70-kg individual, a reasonable dietary level in humans would utilize a safety factor of at least ‘100’ which would decrease exposure to at most 1.5 mg/kg bw/d or 105 mg/d in a 70-kg person, a level of exposure to CAG comparable with possible, albeit not usual, exposure from Radix Astragali in TCMs. Predictably, natural concentrations of CAG and ASTs in TCM formulations vary with the roots used. Root concentrations, in turn, are dependent on source species, environmental factors during culture, and age and season at harvest (Ma et al., 2002; Shibata et al., 1996). An example of a high endogenous concentration was estimated from A. membranaceus roots (1 kg) obtained from a Chinese drugstore in Hangzhou (Yang et al., 2005). After being powdered and extracted in 70% ethanol, 10.96 g of saponin extract were yielded. When analyzed, 83.6% of the extract (equivalent to 9.16 mg/g dry wt in the root) was composed of identifiable ASTs, nearly all of which contained CAG as the aglycone. The CAG content in the root material was estimated to be in the range of 5.34–5.73 mg/g dry wt. Based on the official accepted daily dose of 9–30 g Astragalus root in China (McKenna et al., 2002), dietary exposure to CAG from this source could potentially range from 48–171 mg/d, a range within which ingestion at 105 mg/d falls.

In the scientific literature, CAG is not recognized to exert cardiovascular effects. Indeed, daily exposure to 150 mg/kg bw CAG in the 90-day subchronic toxicity study did not induce cardiovascular effects. Neither the blood pressure measures nor the serum enzymes revealed any statistically significant differences between the dose groups and their respective controls in the main toxicity or recovery studies. As a further indication that CAG did not affect blood pressure, measured values for rats in Groups 5 and 6 in the recovery study (W eek 16) and Groups 1 and 4 in the main toxicity study (Week 12) were comparable by sex. Enzymes AST, CK and LDH were monitored because all are present in myocardial tissue in sufficient quantities that even minor localized damage would result in markedly increased circulating activities. CAG consumption exerted no identifiable effect on the serum activities of these enzymes. In additional support of the negative findings of the cardio-component, no adverse macroscopic or microscopic observations noted for the heart were related to test substance administration. Although in female rats of Groups 3 and 4, absolute and relative heart weights were statistically increased compared with those of the control group, the changes which may have been adaptive were not toxicologically relevant. The changes lacked any kind of clinical or histopathologic correlate, and the Group 4 increases were each intermediate in value to Groups 2 and 3. Further, no similar observations were made in recovery study females. In addition, findings for the FOB conducted during the in-life phase of the main toxicity study were normal and unaffected by CAG administration; neither erratic nor subdued behaviors were reported. Taken all together, under the conditions of this study, CAG was not found to induce cardiac effects.

Daily ingestion of CAG at up to 150 mg/kg bw/day via oral gavage was well-tolerated by the rats in the 13-week subchronic toxicity study with 4-week recovery. No biologically relevant effects attributable to the administration of CAG were identified from the in-life observations, ophthalmology, urinalysis, hematology, clinical chemistry, organ weights, gross pathology, or histopathology in main toxicity or recovery group animals. Although statistical significance was shown for several parameters, each change was only of sporadic incidence; did not demonstrate a dose-dependent relationship; was also observed in the control group; was not correlated to other clinical and/or histopathologic change; lacked toxicological relevance; and/or was within the historically observed ranges in the age and strain of rat used in the study (Pettersen et al., 1996; Derelanko, 2000). In addition, no adverse cardiac-related effects as monitored by changes in blood pressure, selected cardiac enzymes, and gross and histopathologic examination were identified in either sex of any dose group in the main toxicity or recovery phases.

5. CONCLUSION

as demonstrated by the findings of the 13-week subchronic repeated oral dose study in rats with a 4-week recovery phase and three genotoxicity assays, the daily administration of CAG was well-tolerated in the rat and did not induce any toxic or genotoxic effect. The oral no-observed-adverse-effect level NOAEL of CAG was 150 mg/kg bw/day, the highest dose tested in male and female rats.

References

Anderson, D.M.W., 1989. Evidence for the safety of gum tragacanth (Asiatic Astragalus spp.) and modern criteria for the evaluation of food additives. Food Addit. Contam. 6, 1–12.

Bedir, E., Calis, I., Aquino, R., Piacente, S., Pizza, C., 1998a. Cycloartane triterpene glycosides from the roots of Astragalus brachypterus and Astragalus microcephalus. J. Nat. Prod. 61, 1469–1472.

Bedir, E., Calis, I., Zerbe, O., Sticher, O., 1998b. Cyclocephaloside I: A novel cycloartane-type glycoside from Astragalus microcephalus. J. Nat. Prod. 61, 503–505.

Bedir, E., Calis, I., Aquino, R., Piacente, S., Pizza, C., 1999a. Secondary metabolites from the roots of Astragalus trojanus. J. Nat. Prod. 62, 563–568.

Bedir, E., Calis, I., Aquino, R., Piacente, S., Pizza, C., 1999b. Trojanoside H: A cycloartane-type glycoside from the aerial parts of Astragalus trojanus. Phytochemistry 51, 1017–1020.

Bedir, E., Calis, I., Dunbar, C., Sharan, R., Buolamwini, J.K., Khan, I.A., 2001a. Two novel cycloartane-type triterpene glycosides from the roots of Astragalus prusianus. Tetrahedron 57, 5961–5966.

Bedir, E., Tatli, I.I., Calis, I., Khan, I.A., 2001b. Trojanosides I-K: New cycloartane-type glycosides from the aerial parts of Astragalus trojanus. Chem. Pharm. Bull. 49,1482–1486.

Castillo, C., Valencia, I., Reyes, G., Hong, E., 1993. 3-Nitropropionic acid, obtained from Astragalus species, has vasodilator and antihypertensive properties. Drug Dev. Res. 28, 183–188.

Chang, H.M., But, P.P.H. (Eds.), 1987. Pharmacology and Applications of Chinese Materia Medica, vol. 2. World Scientific Publishing, Singapore. de Jesus, B.B., Schneeberger, K., Vera, E., Tejera, A., Harley, C.B., Blasco, M.A., 2011. The telomerase activator TA-65 elongates short telomeres and increases health span of adult/old mice without increasing cancer incidence. Aging Cell 10, 604–621.

Derelanko, M.J., 2000. Clinical pathology. In: Toxicologist's Pocket Handbook. CRC Press, New York, pp. 97–122.