NTP
National Toxicology Program

The Toxicity and Pathology of Dietary Herbals, Botanicals & Supplements

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Presentation Outline

I. Herbal medicine use in the U. S.

II. NTP 2-Year cancer studies of herbal medicines
- Herbal medicine studies with carcinogenic activity
- Herbal medicine without clear evidence of carcinogenic activity

III. NTP Studies of Cardiotoxicity
- Ephedrine/Caffeine Studies

IV. NTP Herbal medicine studies – treatment-related lesions (A. Nyska)

I. Herbal Medicine Use in the U. S.

Herb	Use
Goldenseal	Skin disease, ulcers, colds, and other infections
Ginkgo biloba extract	Asthma, bronchitis, fatigue, memory loss
Kava kava	Anxiety, insomnia, menopausal symptoms
Aloe Vera whole leaf nondecolorized extract	In laxatives
Milk thistle extract	Lower cholesterol levels, Proposed anticancer agent
Tumeric Oleoresin	Proposed anticancer agent
Ginseng	Proposed anticancer agent
Ephedrine	In weight loss products

Herbal Medicines are Complex Mixtures

- Milk thistle – Flavolignan – silymarin, silidyanin, & silychristin
  - Inhibit CYP activity
- Curcumin – Major component in tumeric oleoresin
  - Inhibit CYP activity
- Gingseng – Gingeosides
  - Inhibit CYP activities
- Ginkgo – Terpenoids and flavonoids
  - Inhibit CYP activities

Chen *et al.*, Current Medicinal Chemistry 2011; 18:3190

FDA Guidelines

- 1994 – Dietary Supplement Health and Education Act of 1994 (DSHEA) which defines the term "dietary supplement”
  - A dietary supplement
    - is ingested
    - supplements the diet
    - not represented as a conventional food or as a sole item of a meal or the diet, and contains a "dietary ingredient"
  - "dietary ingredients”
    - may include vitamins, minerals, herbs or other botanicals, amino acids, and dietary substances such as enzymes
    - also can be metabolites, constituents, extracts, concentrates, or combinations of the preceding types of ingredients
  - DSHEA placed dietary supplements in a special category under the general umbrella of "foods," except where the product meets the drug definition
  - http://www.fda.gov/NewsEvents/Testimony/ucm115163.htm

FDA Guidelines

- Under DSHEA, a dietary supplement is adulterated if, among other things, it or any of its ingredients presents "a significant or unreasonable risk of illness or injury" when used as directed on the label, or under normal conditions of use if there are no directions. FDA bears the burden of proof to show that a product or ingredient presents such a risk. In addition, the Secretary of Health and Human Services (HHS) has the authority to declare that a dietary supplement or dietary ingredient poses an "imminent hazard" to public health or safety.

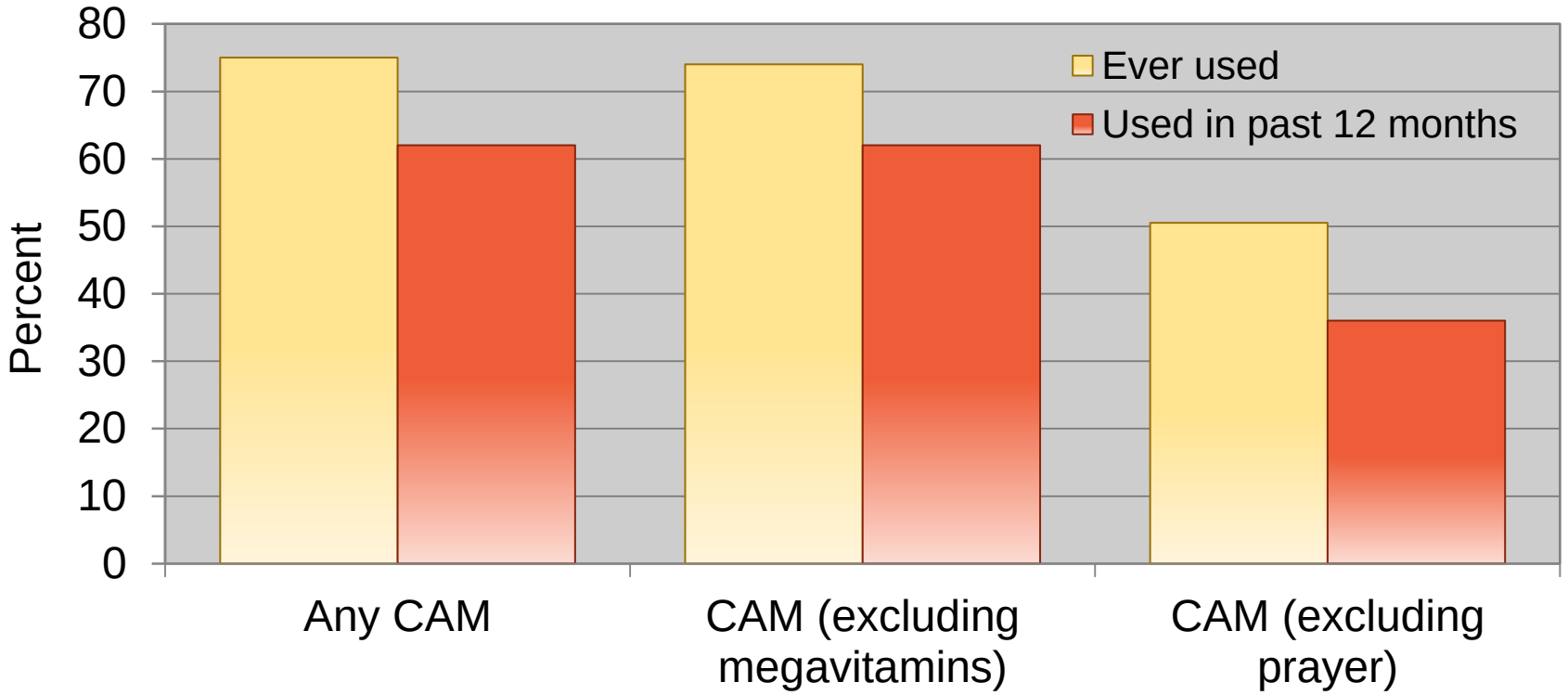
- http://www.fda.gov/NewsEvents/Testimony/ucm115163.htm

Center for Disease Control and Prevention National Health and Nutrition Examination Survey

- The dietary supplements section provides personal interview data on the use of supplements and herb in the U. S.
- http://www.cdc.gov/nchs/nhanes/nhanes2003-2004/diet03_04.htm

Center for Disease Control and Prevention National Health and Nutrition Examination Survey

Age-adjusted percent of adults who have used complementary and alternative medicine: United States, 2002



Note: CAM is complementary and alternative medicine.
Data Source: National Health Interview Survey, 2002.

II. NTP 2-Year Cancer Studies of Herbal Medicines

- **Liver carcinogens**
  - Goldenseal – rats and mice (TR 562)
  - Ginkgo biloba extract – mice (TR 578)
  - Kava kava extract – mice (TR 571)
- **Intestinal carcinogen**
  - Aloe vera whole leaf nondecolorized extract – rats (TR 577)
    (Noncolorized whole leaf extract *Aloe barbadensis* Miller)
- **No or equivocal evidence for carcinogenic activity**
  - Milk thistle extract – rats and mice (TR 565)
  - Tumeric oleoresin – rats and mice (TR 427)
  - Ginseng – rats and mice (TR 567)

II. NTP 2-Year Cancer Studies of Herbal Medicines

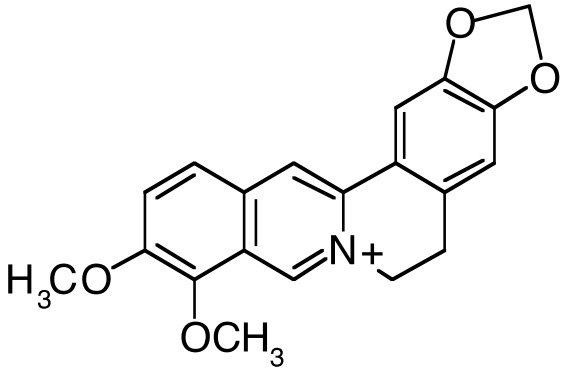
- **Liver carcinogens**
  - Goldenseal – *J. Dunnick & J. Peckham, NIEHS/NTP*
  - Ginkgo biloba extract – *C. Rider, P. Chan, A. Nyska, NIEHS/NTP*
  - Kava kava extract – *M. Behl, P. Chan, A. Nyska, NIEHS/NTP*
- **Intestinal carcinogen**
  - Aloe vera whole leaf nondecolorized extract – *M. Boudreux & F. Beland, NCTR/FDA/NTP*
- **No or equivocal evidence for carcinogenic activity**
  - Milk thistle extract – *J. Dunnick & A. Nyska, NIEHS/NTP*
  - Tumeric oleoresin – *J. Dunnick & R. Sills, NIEHS/NTP*
  - Ginseng – *P. Chan & J. Peckham, NIEHS/NTP*
- **Heart toxicity**
  - Ephedrine/caffeine – *J. Dunnick & A. Nyska, NIEHS/NTP*

Goldenseal – TR 562 Feed 0, 3,000, 9,000, 25,000 ppm

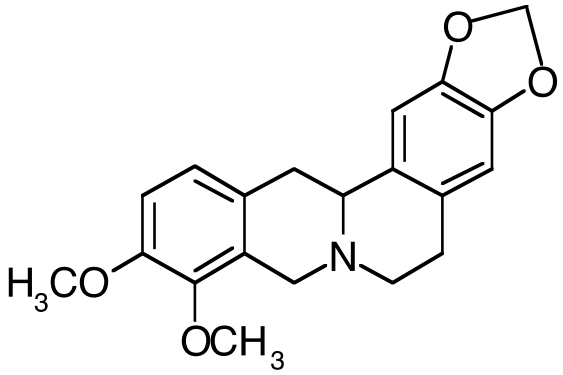
- **Male F344/N rats**: **clear** evidence of carcinogenic activity
  - Hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined)
- **Female F344/N rats**: **clear** evidence of carcinogenic activity
  - Hepatocellular adenoma
- **Male B6C3F1 mice**: **some** evidence of carcinogenic activity
  - Hepatoblastoma and multiple hepatocellular adenoma
- **Female B6C3F1 mice: no** evidence of carcinogenic activity

- Goldenseal – negative in gentox tests
- Major active component: Berberine – positive in gentox test; topisomerase inhibition (enzyme essential in DNA repair processes)

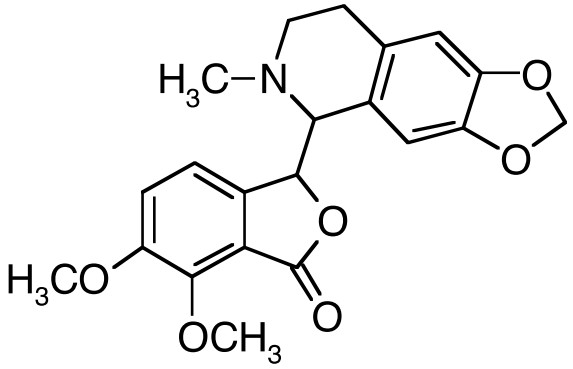
Goldenseal Active Ingredients



Berberine



Canadine



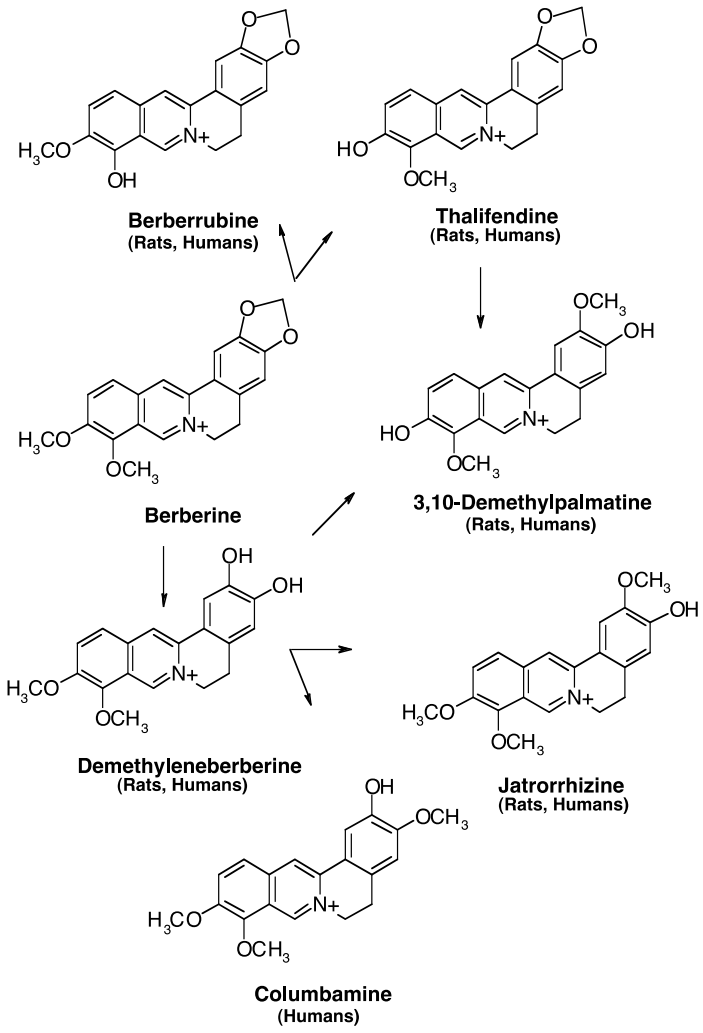
Hydrastine

Goldenseal – 2-year Dietary Feeding Study in F344/N Rats and B6C3F1 Mice

Dose (ppm)	0	3000	9000	25,000
Male rats				
Hepatocellular adenoma, multiple	0	0	0	2
Hepatocellular adenoma (includes multiple)	1**[a]	1	2	10**[b]
Hepatocellular carcinoma	0	0	0	1
Hepatocellular adenoma or carcinoma	1**	1	2	11**
Female rats				
Hepatocellular adenoma	0**	0	1	8**
Male mice				
Hepatoblastoma (multiple)	0	0	0	2
Hepatoblastoma (includes multiple)	1*	2	1	6
Hepatocellular adenoma (multiple)	3	5	11*	18**
Hepatocellular adenoma (includes multiple)	22*	16	23	29

[a]Trend statistic [b]Pairwise statistic *$p \leq 0.05$ **$p \leq 0.01$ N=50

Berberine Metabolites in Rats and Humans



Ginkgo Biloba Extract – 2-year Oral Gavage (Corn Oil) Study in F344/N Rats (0, 100, 300, 1,000 mg/kg) and B6C3F1 Mice (0, 200, 600, 2,000 mg/kg)

- **Male F344/N rats**: **some** evidence of carcinogenic activity
  - Thyroid gland follicular cell adenoma
  - Mononuclear cell leukemia & hepatocellular adenoma may have been related to treatment
- **Female F344/N rats**: **some** evidence of carcinogenic activity
  - Thyroid gland follicular cell neoplasms
  - Respiratory epithelium adenoma may have been related to treatment
- **Male B6C3F1 mice**: **clear** evidence of carcinogenic activity
  - Hepatocellular carcinoma and hepatoblastoma
  - Thyroid follicular cell adenoma were also related to treatment
- **Female B6C3F1 mice**: **clear** evidence of carcinogenic activity
  - hepatocellular adenoma and carcinoma, hepatoblastoma
- Positive in Salmonella assays with/without activation

Ginkgo Components

- Terpene trilatones and flavonal glycosides
- Ginkgolic acids shown to mutagenic and cytotoxic components

Ginkgo Biloba Extract – 2-year Oral Gavage (Corn Oil) Study in F344/N Rats and B6C3F1 Mice

Dose (mg/kg)	0	200	600	2000
Male mice				
Hepatoblastoma	3**[a]	28**	36**	38**[b]
Hepatocellular carcinoma	22**	31*	41**	47**
Hepatocellular adenoma or carcinoma	39**	46**	46**	49**
Female mice				
Hepatoblastoma	1**	1	8**	11**
Hepatocellular carcinoma	9**	10	15	44**
Hepatocellular adenoma or carcinoma	20**	39**	41**	49**

[a]Trend statistic [b]Pairwise statistic *$p \leq 0.05$ **$p \leq 0.01$ N=50

Nonneoplastic and Neoplastic Lesions in Thyroid of Rats in the 2-years Gavage Study of Ginkgo Biloba Extract (N=50)

	Control	100 mg/kg	300 mg/kg	1000 mg/kg
Male rats				
Follicular cell hypertrophy	13(1.0)	37**(1.2)	41**(1.3)	41**(1.8)
Follicular cell hyperplasia	0	7**(1.3)	9**(2.0)	5*(2.8)
Follicular cell adenoma	2	1	3	5
Female rats				
Follicular cell hypertrophy	15(1.0)	41**(1.0)	45**(1.1)	48**(2.0)
Follicular cell adenoma	0	0	3	1
Follicular cell carcinoma	0	0	1	1

*significantly different ($p \leq 0.05$) from vehicle control group by the Poly-3 test
**$p \leq 0.01$

Nonneoplastic and Neoplastic Lesions in Thyroid Mice in the 2-years Gavage Study of Ginkgo Biloba Extract (N=50)

	Control	100 mg/kg	300 mg/kg	1000 mg/kg
Male mice				
Follicular cell hypertrophy	2(1.0)	0	2(1.5)	38**(1.2)
Follicular cell hyperplasia	2(1.0)	1(1.0)	7(1.1)	25**(1.4)
Follicular cell adenoma	0	0	2	2
Female rice				
Follicular cell hypertrophy	1(3.0)	5(1.4)	9*(1.0)	39**(1.0)

*significantly different ($p \leq 0.05$) from vehicle control group by the Poly-3 test
**$p \leq 0.01$

Nonneoplastic Lesions in the Nose of Rats in the 2-years Gavage Study of Ginkgo Biloba Extract (N=50)

	Control	100 mg/kg	300 mg/kg	1000 mg/kg
Male rats				
Olfactory epithelium, atrophy	1(1.0)	26**(1.3)	37**(1.6)	31**(2.2)
Nerve, olfactory epithelium, atrophy	0	17**(1.4)	14**(2.1)	23**(2.5)
Olfactory epithelium, respiratory metaplasia	9(1.3)	30**(1.5)	40**(2.0)	32**(1.5)
Chronic active inflammation	33 (1.2)	32(1.3)	38(1.9)	46**(2.2)
Female rats				
Olfactory epithelium, atrophy	0	18**(1.1)	25**(1.6)	37**(2.1)
Nerve, olfactory epithelium, atrophy	0	15**(1.1)	22**(1.6)	33**(2.2)
Olfactory epithelium, respiratory metaplasia	8(1.3)	4 (1.3)	32**(2.0)	37**(2.5)
Chronic active inflammation	22(1.0)	1691.2)	26(1.5)	38**(1.9)
Respiratory epithelium, adenoma	0	0	2	0

*significantly different ($p \leq 0.05$) from vehicle control group by the Poly-3 test
**$p \leq 0.01$

Nonneoplastic Lesions in the Nose of Mice in the 2-years Gavage Study of Ginkgo Biloba Extract (N=50)

	Control	100 mg/kg	300 mg/kg	1000 mg/kg
Male mice				
Olfactory epithelium, hyaline droplet accumulation	18(1.4)	16(1.9)	15(1.8)	28*(1.8)
Olfactory epithelium, pigmentation	0	1(1.0)	3(1.0)	13**(1.1)
Female mice				
Olfactory epithelium, hyaline droplet accumulation	5(1.0)	3(1.7)	12(1.2)	17**(1.6)
Olfactory epithelium, pigmentation	0	1(1.0)	6*(1.5)	13**(1.2)

*significantly different ($p \leq 0.05$) from vehicle control group by the Poly-3 test
**$p \leq 0.01$

Kava Kava Extract – TR 571 2-year Oral Gavage (Corn Oil) Study in F344/N Rats (0, 100, 300 1000 mg/kg) and B6C3F1 Mice (0, 250, 500, 1,000 mg/kg)

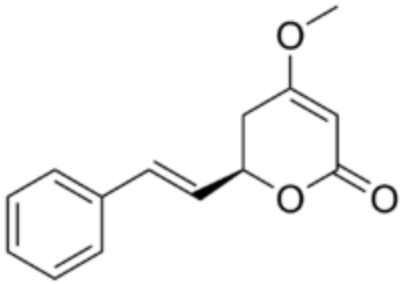
- **Male F344/N rats: <u>equivocal</u>** evidence of carcinogenic activity
  - Marginal increase in testicular adenomas
- **Female F344/N rats**: **<u>no</u>** evidence of carcinogenic activity
- **Male B6C3F1 mic**e: **<u>clear</u>** evidence of carcinogenic activity
  - Hepatocellular tumors and hepatoblastomas
- **Female B6C3F1 mice**: **<u>some</u>** evidence of carcinogenic activity
  - Hepatocellular adenomas and carcinomas (combined)
- Negative in Salmonella assay

Kava Kava – 2-year Oral Gavage (Corn Oil) Study in F344/N Rats and B6C3F1 Mice

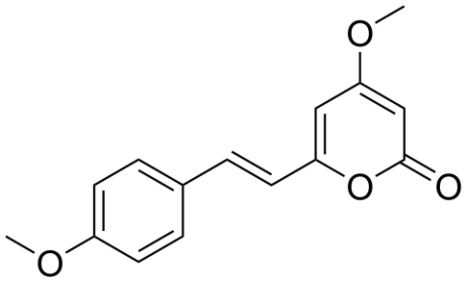
Dose (mg/kg)	0	250	500	1000
Male mice				
Hepatoblastoma	0**[a]	4	9**	12**[b]
Hepatocellular carcinoma	20	18	26	20
Hepatocellular carcinoma or hepatoblastoma	20	21	30	25
Female mice				
Hepatocellular carcinoma	3	13**	8	8
Hepatocellular adenoma or carcinoma	10	21*	20*	13

[a]Trend statistic [b]Pairwise statistic *$p \leq 0.05$ **$p \leq 0.01$ N=50

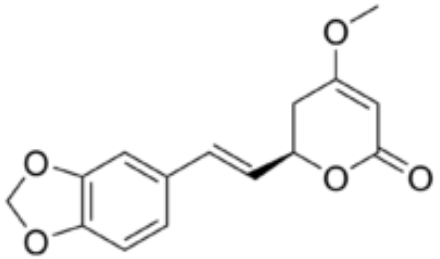
Kava Kava Extract Comprises 30% Total Kavalactones – Consisting of 6 Major Kavalactones



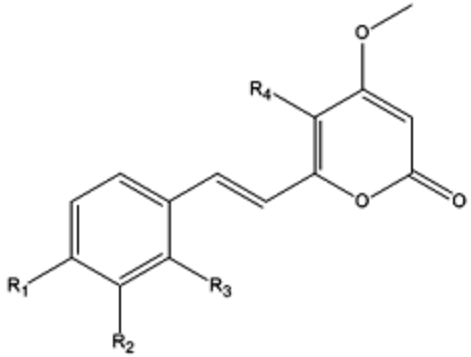
Kavain



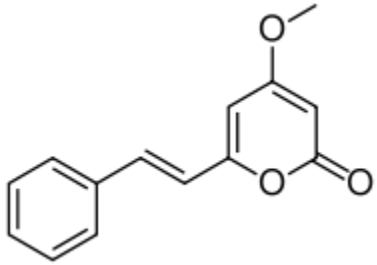
Yangonin



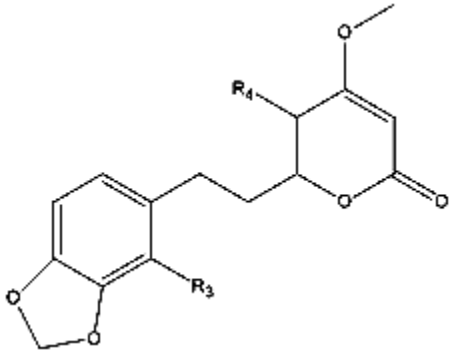
Methysticin



7,8 Dihydrokavain



Desmethoxyyangonin/
5,6 dehydrokavain



Dihydromethysticin

R1, R2, R3, R4 = H

Aloe Vera – TR 577 Noncolorized Whole Leaf Extract
Drinking Water 0, 500, 1,000, 1,500 ppm

- **Male F344/N rats: clear** evidence of carcinogenic activity
  - Adenoma and carcinoma of the large intestine
- **Female F344/N rats: clear** evidence of carcinogenic activity
  - Adenoma and carcinoma of the large intestine

- **Male B6C3F1 mice: no** evidence of carcinogenic activity
- **Female B6C3F1 mice**: **no** evidence of carcinogenic activity
- Aloe emodin positive in Salmonella assays

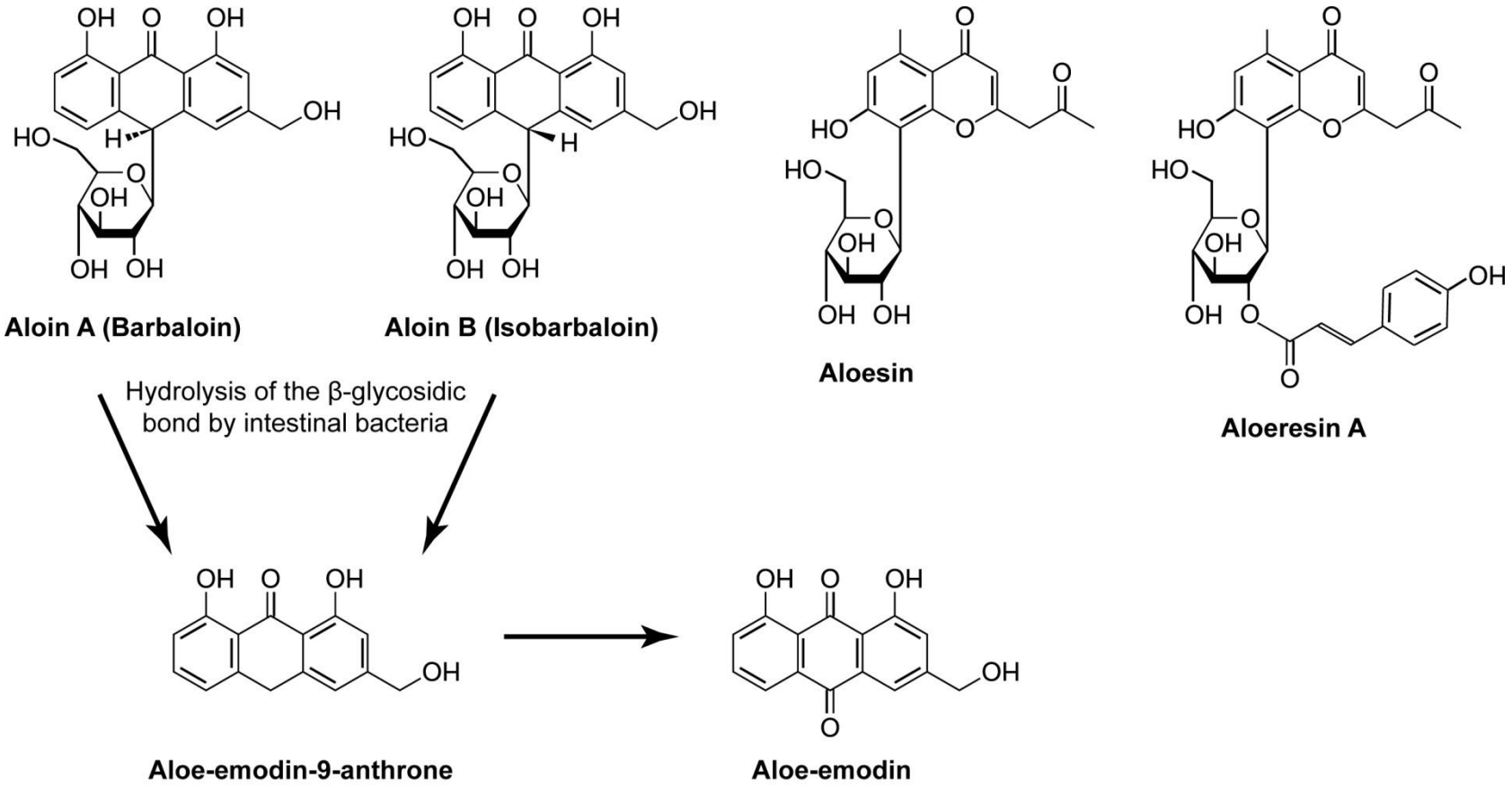
Aloe Vera – 2-year Drinking Water Study in F344/N Rats and B6C3F1 Mice

Dose (ppm)	0%	0.5%	1.0%	1.5%
Male rats				
Large Intestinal adenoma or carcinomas	0*[a]	0	28**	31**[b]
Female rats				
Large Intestinal adenoma/carcinoma	0**	0	8**	15**
Male and female mice	No evidence of carcinogenic activity			

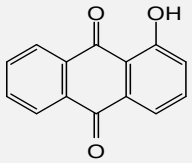
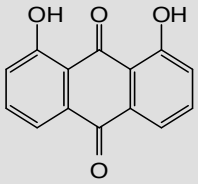
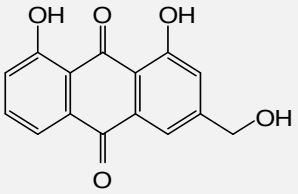
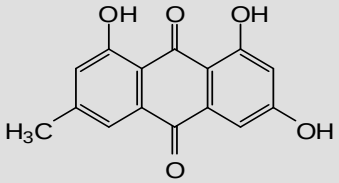
[a]Trend statistic [b]Pairwise statistic *$p \leq 0.05$ **$p \leq 0.01$ N=48

Aloe Active Ingredient – Aloin A & B – Metabolized to Aloe Emodin in the Intestinal Tract

Structures of Aloe vera Latex-derived Anthraquinone *C*-glycosides, Anthrone, and Anthraquinone



Intestinal Lesions/Tumors Occur in Rat (Drinking Water or Feed) Bioassays of Hydroxyanthraquinones or Herbals Containing Anthraquinones

Bioassay/Representative Anthraquinone	Cancer Study in Mice	Cancer Study in Rats	Reference
1-Hydroxyanthraquinone 	No study	ACI/N rats Intestinal tumors (also liver and stomach tumors) (feed study)	Mori *et al.*, 1990
Danthron 1,8-dihydroxyanthraquinone 	C3H/HeN mice Intestinal hyperplasia (no tumors) (feed study)	ACI/N rats Intestinal tumors (feed study)	Mori *et al.*, 1986 (mice) Mori *et al.*, 1985 (rats)
Aloe vera leaf extract/ Aloe emodin 1,8-dihydroxy-3-hydroxymethyl-anthraquinone 	B6C3F1 mice Intestinal hyperplasia (no tumors) (drinking water study)	F344/N rats Intestinal tumors (drinking water)	NTP TR 577
Emodin 1,3,8-trihydroxy-6-methylantharquinone 	B6C3F1 mice No intestinal lesions or tumors (feed study)	F344/N rats No intestinal lesions (feed study)	NTP TR 493

Summary of Point Mutations in Aloe Vera Intestinal Tumors in F344/N Rats

- Point mutations in Kras (codon 13) - 2/12
- Point mutations in Kras (codon 12) - 1/12
- Point mutations in Ctnnb1(exon 2) - 4/12
- No point mutations in p53 (exon 5 -8) - 0/12
- Molecular pathways involved in carcinogenic process – WNT, MAPK, TGF-β

Pandiri *et al.* Aloe vera Non-Decolorized Whole Leaf Extract-Induced Large Intestinal Tumors in F344 Rats Share Similar Molecular Pathways with Human Sporadic Colorectal Tumors, ToxPath 39: 1065-1074, 2011

Milk Thistle – TR 565
Feed 0, 12,500, 25,000, 50,000 ppm

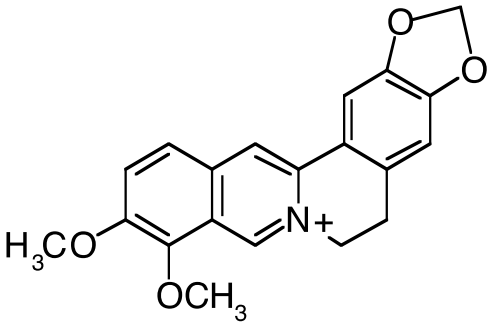
- **Male F344/N Rats: No** evidence of carcinogenic activity
- **Female F344/N Rats: No** evidence of carcinogenic activity
- **Male B6C3F1 Mice: No** evidence of carcinogenic activity
- **Female B6C3F1 Mice: No** evidence of carcinogenic activity
- Milk thistle extract: negative in Salmonella

Milk Thistle Extract – 2-year Dietary Feeding Study in F344/N Rats and B6C3F1 Mice

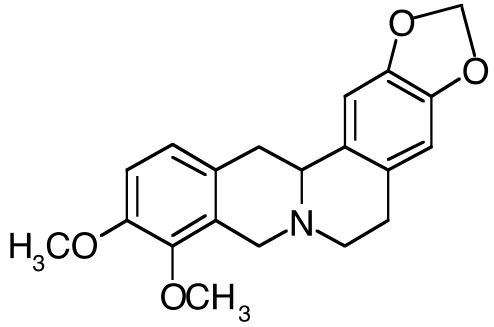
Dose (ppm)	0	12,500	25,000	50,000
Male rats				
Bile duct hyperplasia	50(2.5)**	32(1.0)	27(1.1)**	15(1.0)**
Female rats				
Bile duct hyperplasia	37(1.4**)	10(1.7)**	10(1.3)**	8(1.1**)
Mammary gland fibroadenoma	28**	28	17*	18*
Male mice				
Hepatocellular adenoma/carcinoma	26**	22	16*	8**

[a]Trend statistic [b]Pairwise statistic *$p \leq 0.05$ **$p \leq 0.01$ N=50

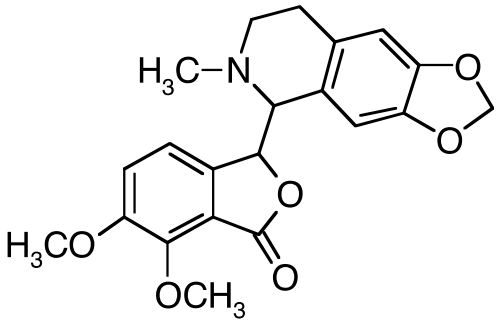
Milk Thistle Extract – Active Ingredients – Metabolites of Active Ingredients Similar in Humans and Animals



Berberine



Canadine



Hydrastine

Tumeric Oleoresin – TR 427
Feed 0, 2,000, 10,000, 50,000 ppm

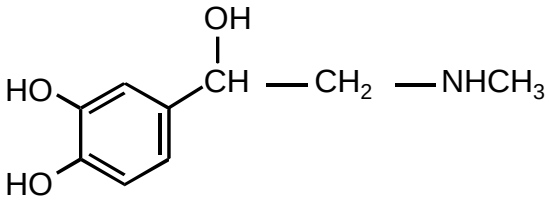
- **Male rats: no evidence** of carcinogenic activity
  - Increased incidences of preputial gland neoplasms
- **Female rats: equivocal evidence** of carcinogenic activity
  - Clitoral gland adenoma
- **Male mice: equivocal evidence** of carcinogenic activity
  - Hepatocellular adenoma
- **Female mice: equivocal evidence** of carcinogenic activity
  - Hepatocellular adenoma
- Tumeric oleoresin – negative in Salmonella

Ginseng – TR 567

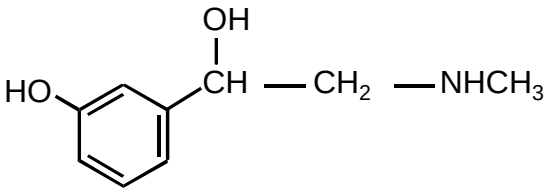
- **Male F344/N rat: no evidence** of carcinogenic activity
- **Female F344/N rat: no evidence** of carcinogenic activity
- **Male B6C3F1 mouse: no evidence** of carcinogenic activity
- **Female B6C3F1 mouse: no evidence** of carcinogenic activity
- Ginseng – negative in Salmonella

III. Cardiotoxicity Studies: Ephedrine/Ephedra (Ma Huang)

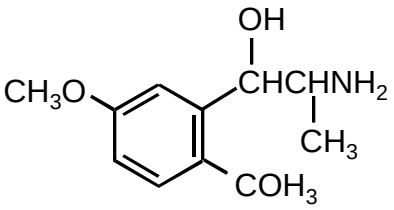
- Ephedrine (active ingredient in Ma Huang) binds to adrenergic receptors
- Ephedrine in combination with caffeine is more toxic than exposure to either compound alone
  - Ephedrine and caffeine in combination alter ion flow (calcium)
- Ephedrine/caffeine exposure increases heart rate and temperature within one hour after a single oral gavage study in rats and mice
- Ephedrine/caffeine exposure cause hemorrhage and necrosis in moribund rats and mice
- Both ephedrine/caffeine and the Herb (Ma Huang)/caffeine exposures cause similar cardiac toxicity



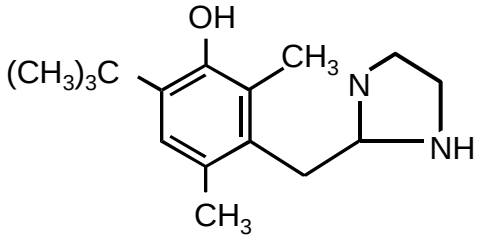
Epinephrine
(non-selective AR agonist)



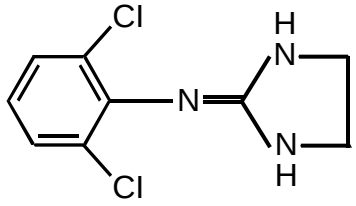
Phenylephrine
(α_1-AR selective agonist)



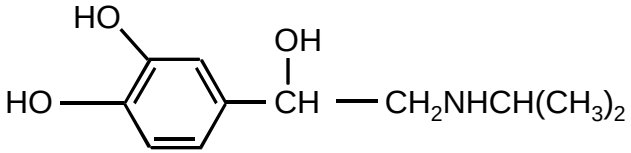
Methoxamine
(α_{1a}-AR selective agonist)



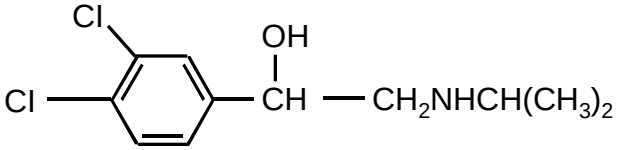
Oxymetazoline
(α_{1a}-AR selective agonist)



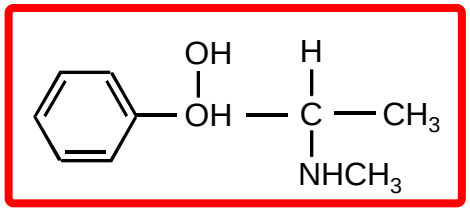
Clonidine
(α_{1a}-AR selective agonist)



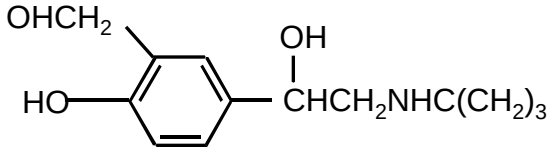
(-)- Isoproterenol
(non-selective β-AR agonist)



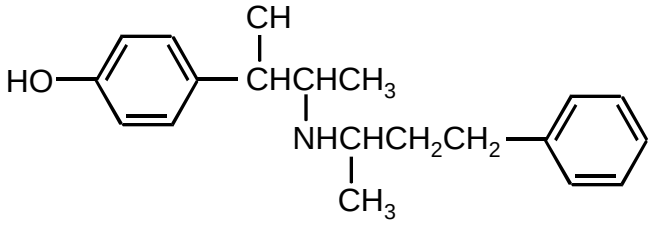
Dichloroisoproterenol
(non-selective β-AR agonist)



Ephedrine
(non-selective β-AR agonist)



Albuterol
(non-selective β-AR agonist)



Nylidrin
(non-selective β-AR agonist)

Waugh et al JBC 275;11698, 2000

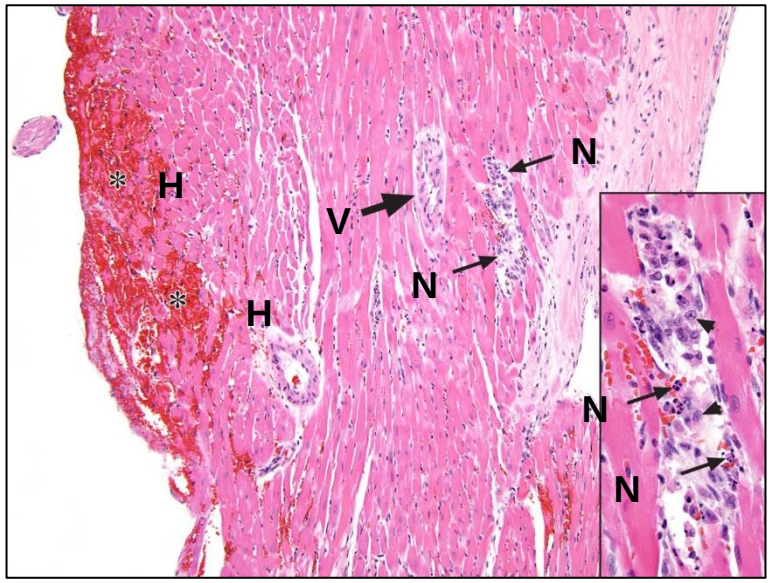
Ephedrine/Caffeine ECG Parameters – 14 Week F344/N Rats One Oral Gavage Dose

Treatment mg/kg	Time Point (hour)	HR Beats/min	QT_c, ms	R-amp, mV	Temp °C
Control	Baseline	355±5	0.112±0.001	0.401±0.015	36.9±0.01
25 Eph + 30 Caff	1	478±5*	0.192±0.004*	0.460±0.028*	39.2±0.6*
25 Eph + 30 Caff	3	485±16*	0.182±0.007*	0.409±0.0371*	38,1±0.2*

*p<0.05

Proposed Mechanism of Ephedrine/Caffeine Heart Toxicity

Oral Ephedrine Exposure → Rapid absorption so that toxic levels are obtained within one hour of dosing → α/β adrenergic agonist
- release of catecholamines
- Calcium influx



Caffeine Release of Intracellular Ca^{++} Stores → Vasoconstriction (V)

↓

Increase HR and QT_c interval

↓

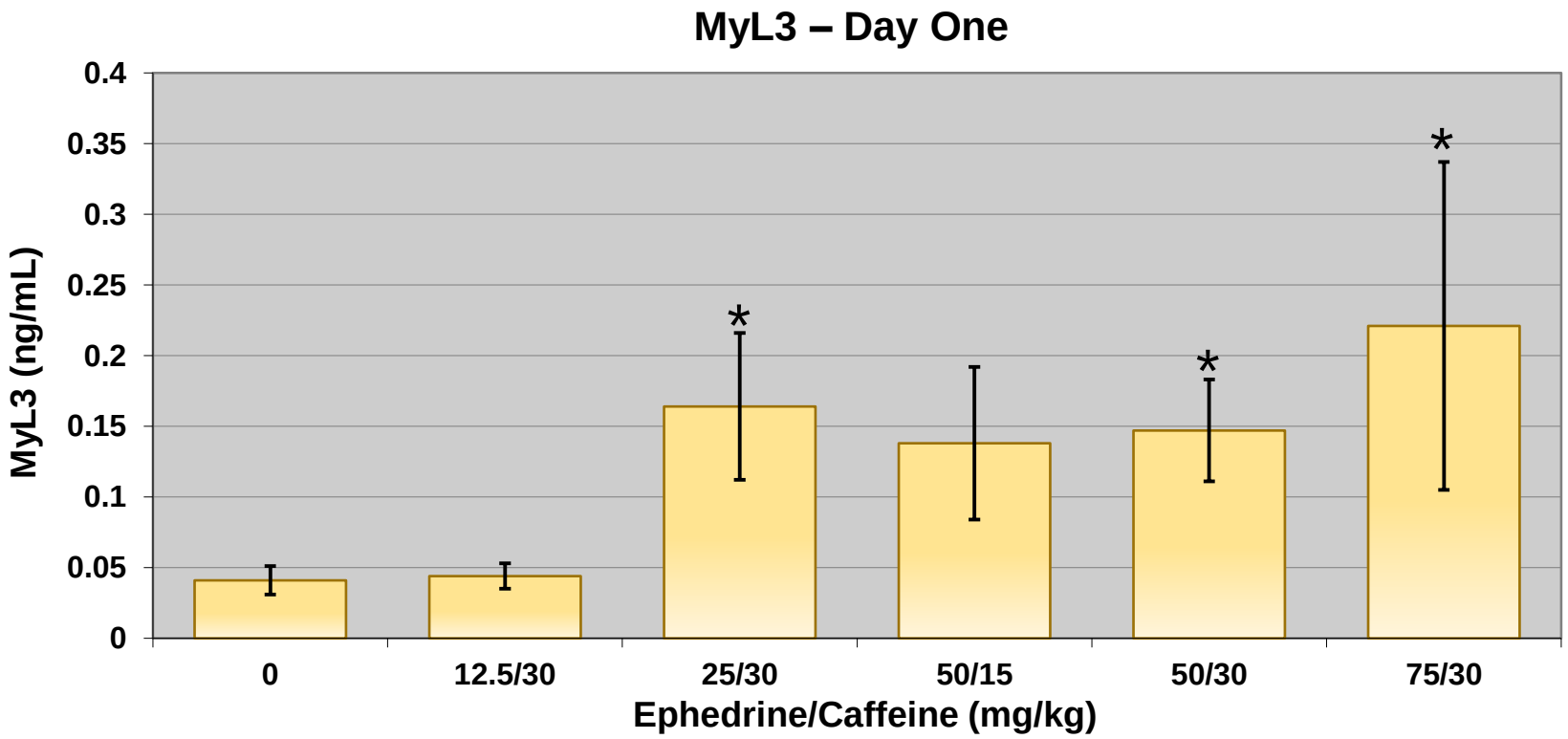
Myocardial ischemia

↓

Necrosis and Apoptosis (N) → Hemorrhage (H) → **Sudden Death**

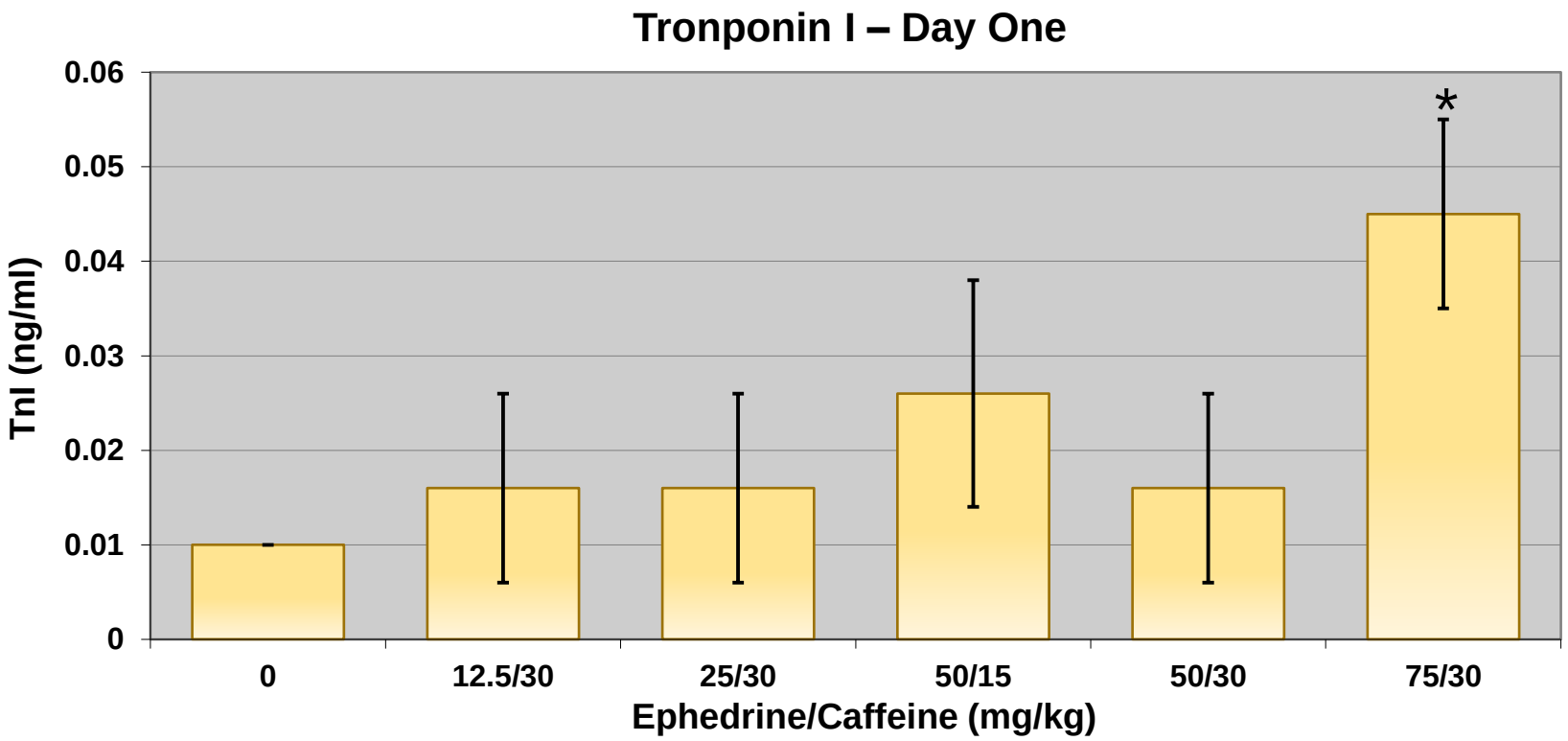
Hemorrhage (H) → Resolution by Inflammation Fibrosis

Serum Biomarkers Detect Ephedrine/Caffeine Cardiotoxicity Even in the Absence of Histopathologic Lesions (Studies in B6C3F1 Mice – One Oral Dose)



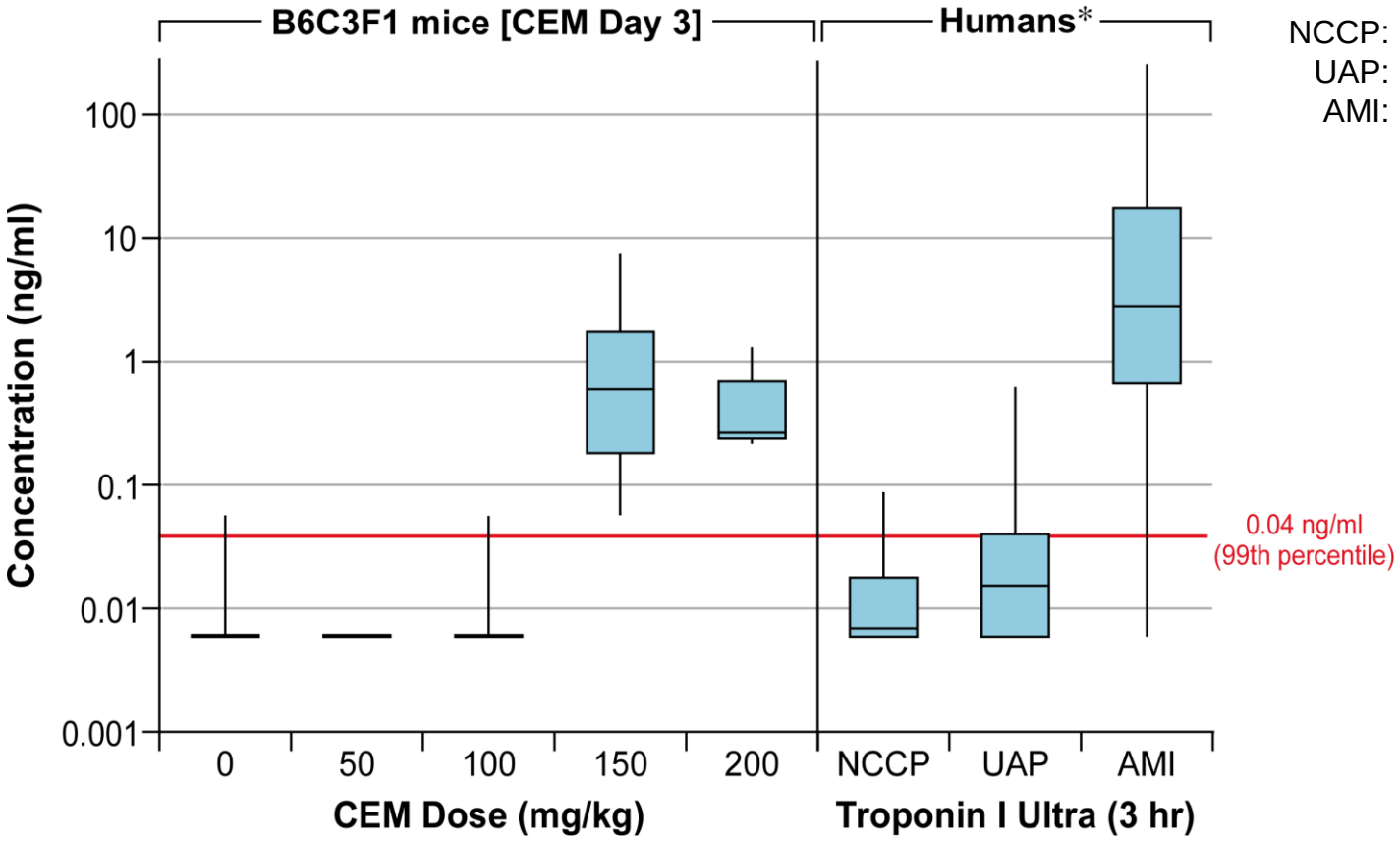
With Dr. G. Travlos & Dr. S. Borgdorf

Serum Biomarkers Detect Ephedrine/Caffeine Cardiotoxicity Even in the Absence of Histopathologic Lesions (Studies in B6C3F1 Mice – One Oral Dose)



With Dr. G. Travlos & Dr. S. Borgdorf

Keller *et al.* Sensitive TNL Assay in Early Diagnosis Infarction NEJM 2009: 361: 868-77 – Human vs. Troponin Levels After Ephedrine/Caffeine



NCCP: new onset of chest pain
UAP: unstable angina pectoris
AMI: acute myocardial infarction

With Dr. G. Travlos & Dr. S. Borgdorf

Summary of NTP Herbal Medicine Findings

- Diverse biologic response among herbs and supplements
- Some are carcinogenic, some are not
- Individual components have biologic activities that help explain the carcinogenic findings
- NIH clinical trials underway for anticancer activity of turmeric (curcumin), milk thistle, ginseng
  - http://www.clinicaltrials.gov/

IV. NTP Herbal Medicine Studies – Treatment-related Lesions (A. Nyska)

- Liver nonneoplastic and neoplastic lesions
- Thyroid nonneoplastic and neoplastic lesions
- Intestinal nonneoplastic and neoplastic lesions
- Heart lesions

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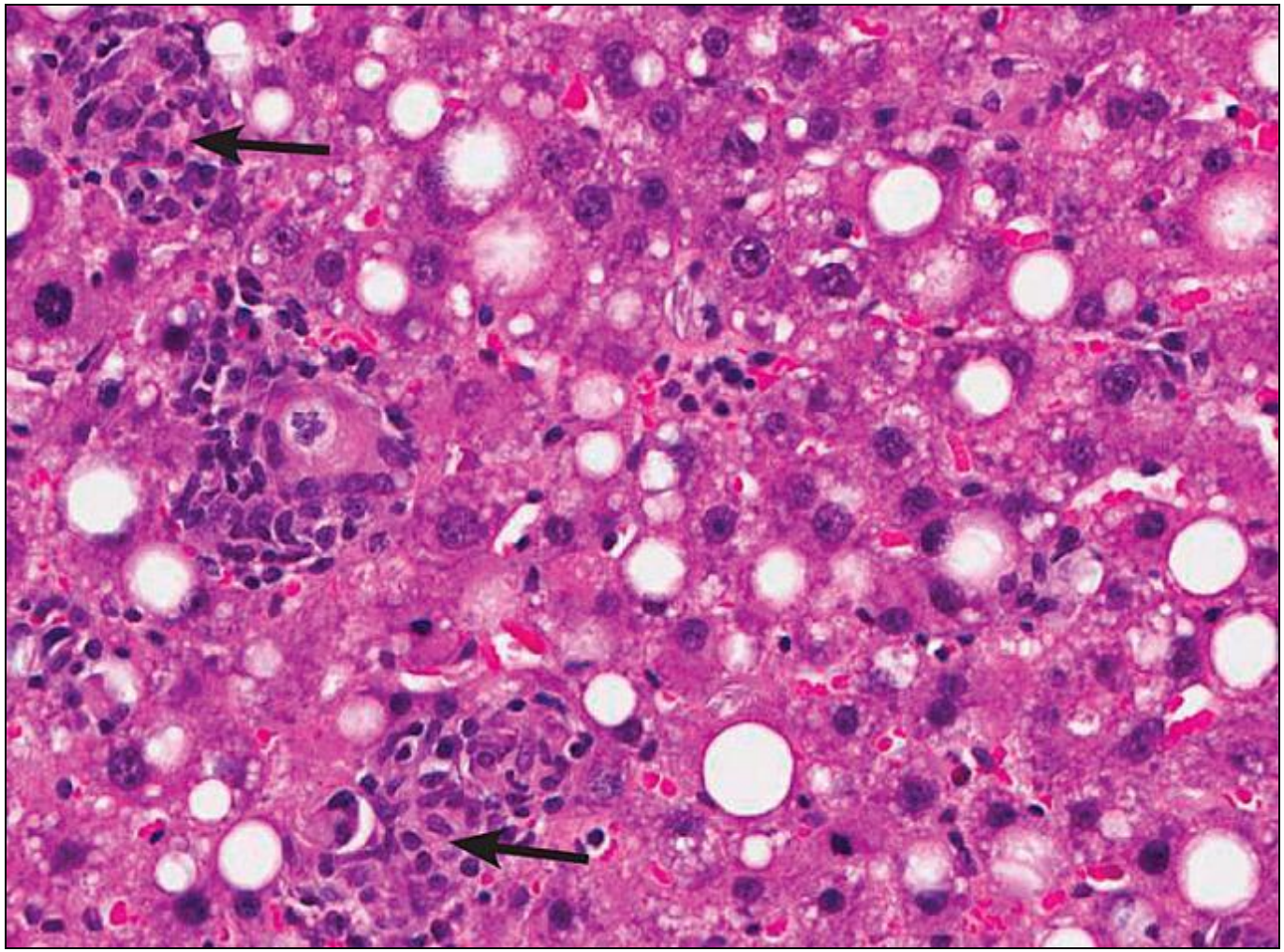
Ginkgo Biloba Extract
NTP Technical Report TR 578

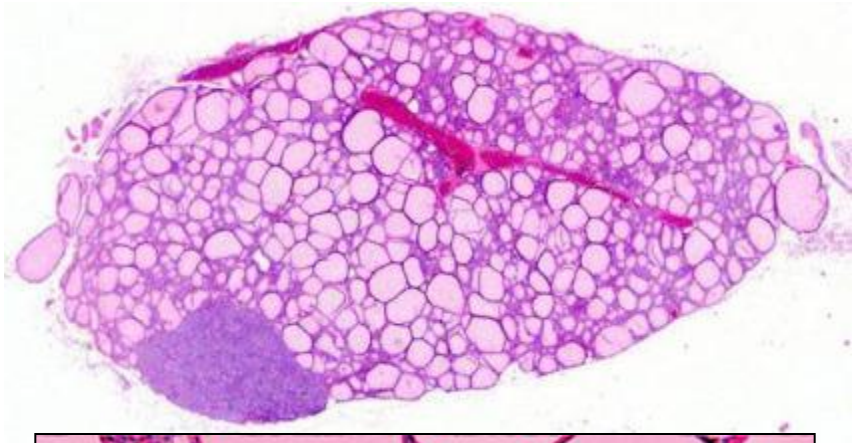
Histopathology Findings
2-Year Studies – Rats

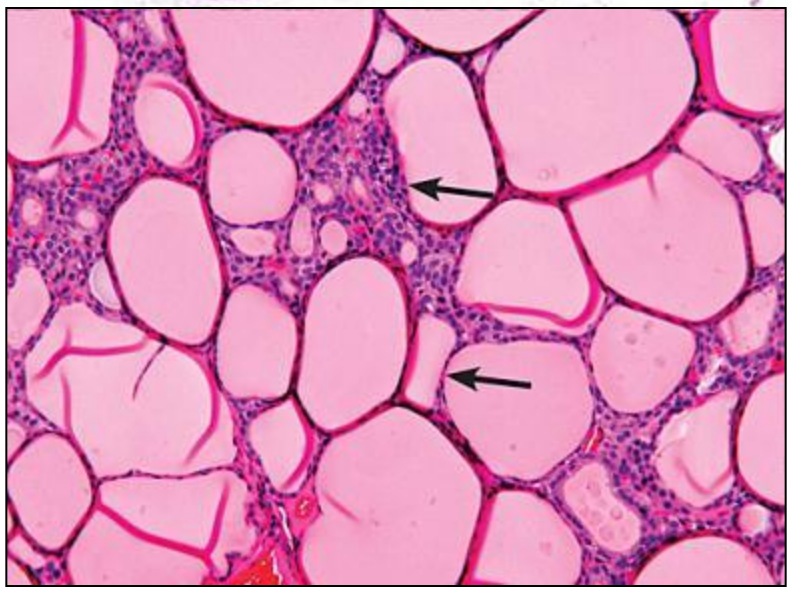
Focal Fatty Change Associated with Microgranulomas in a Female Rat Treated with 1000 mg/kg of Ginkgo Biloba



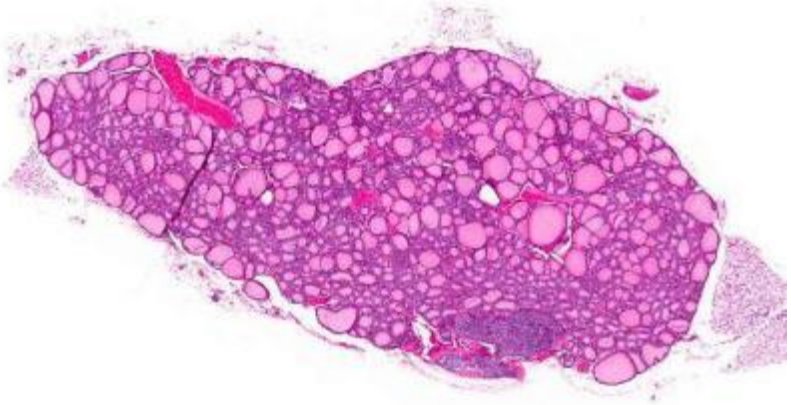
Thyroid Follicular Hypertrophy in a Female Rat Treated for 2 Years with 1000 mg/kg of Ginkgo Biloba, Comparing to the Aspect in a Concurrent Control Animal

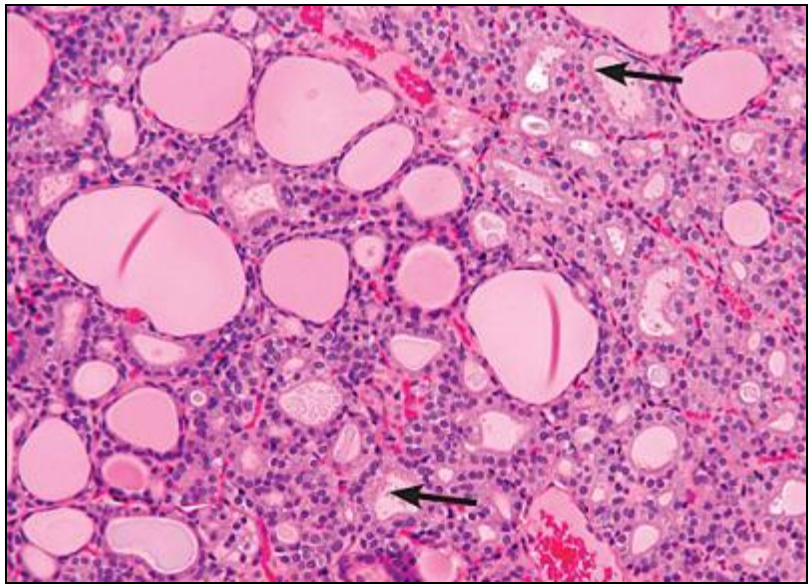
Control Rat



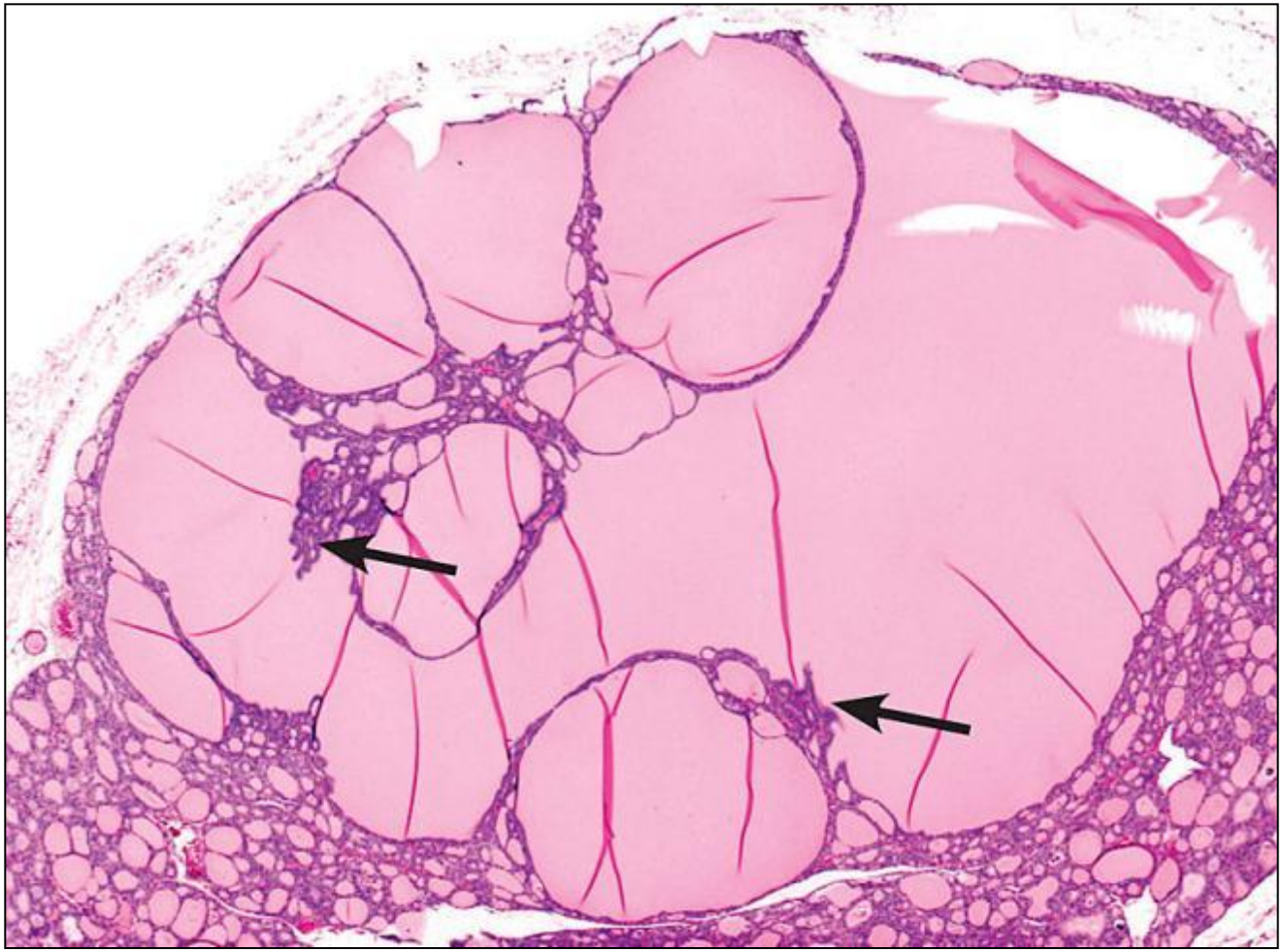


Treated Rat

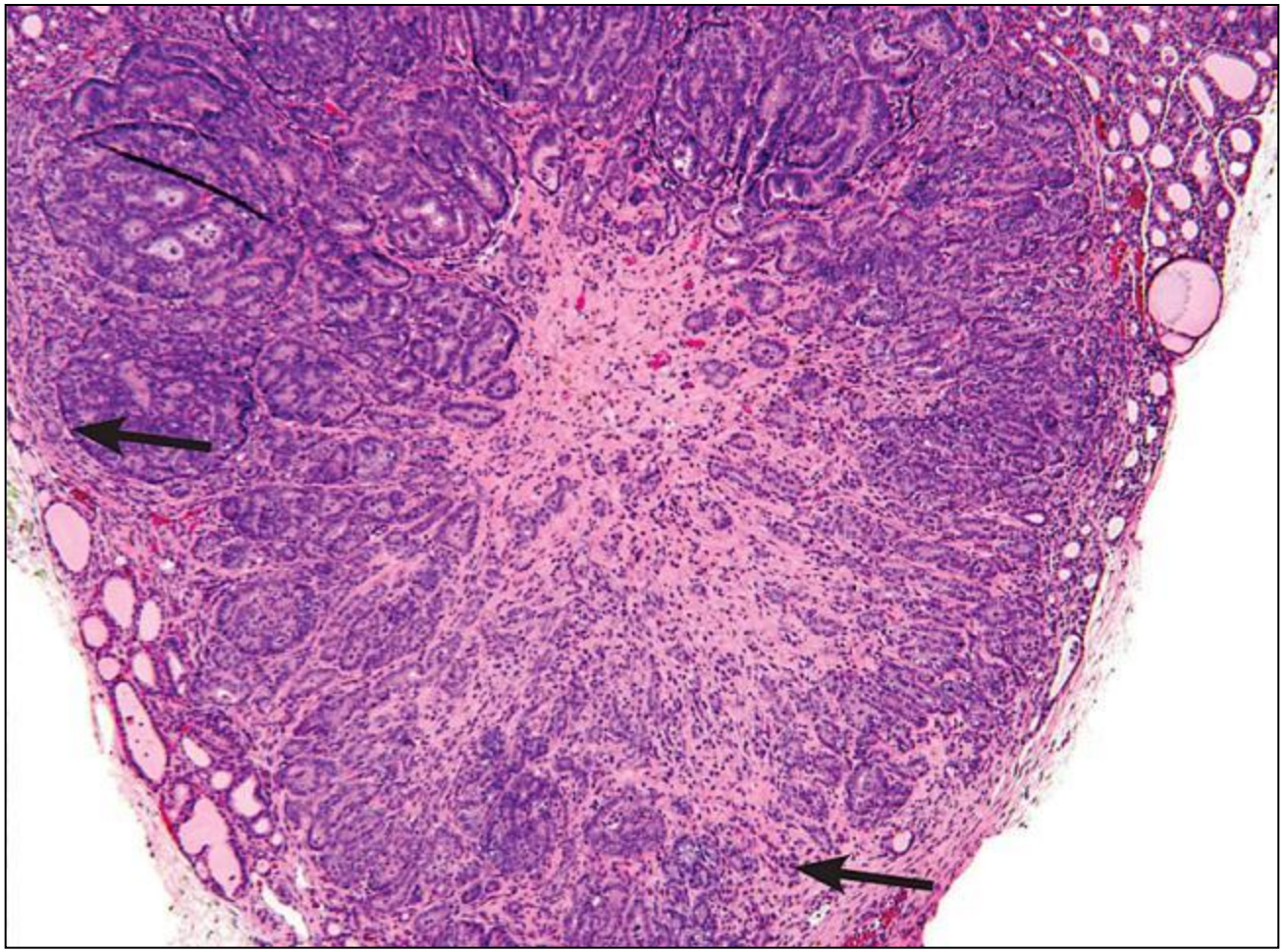




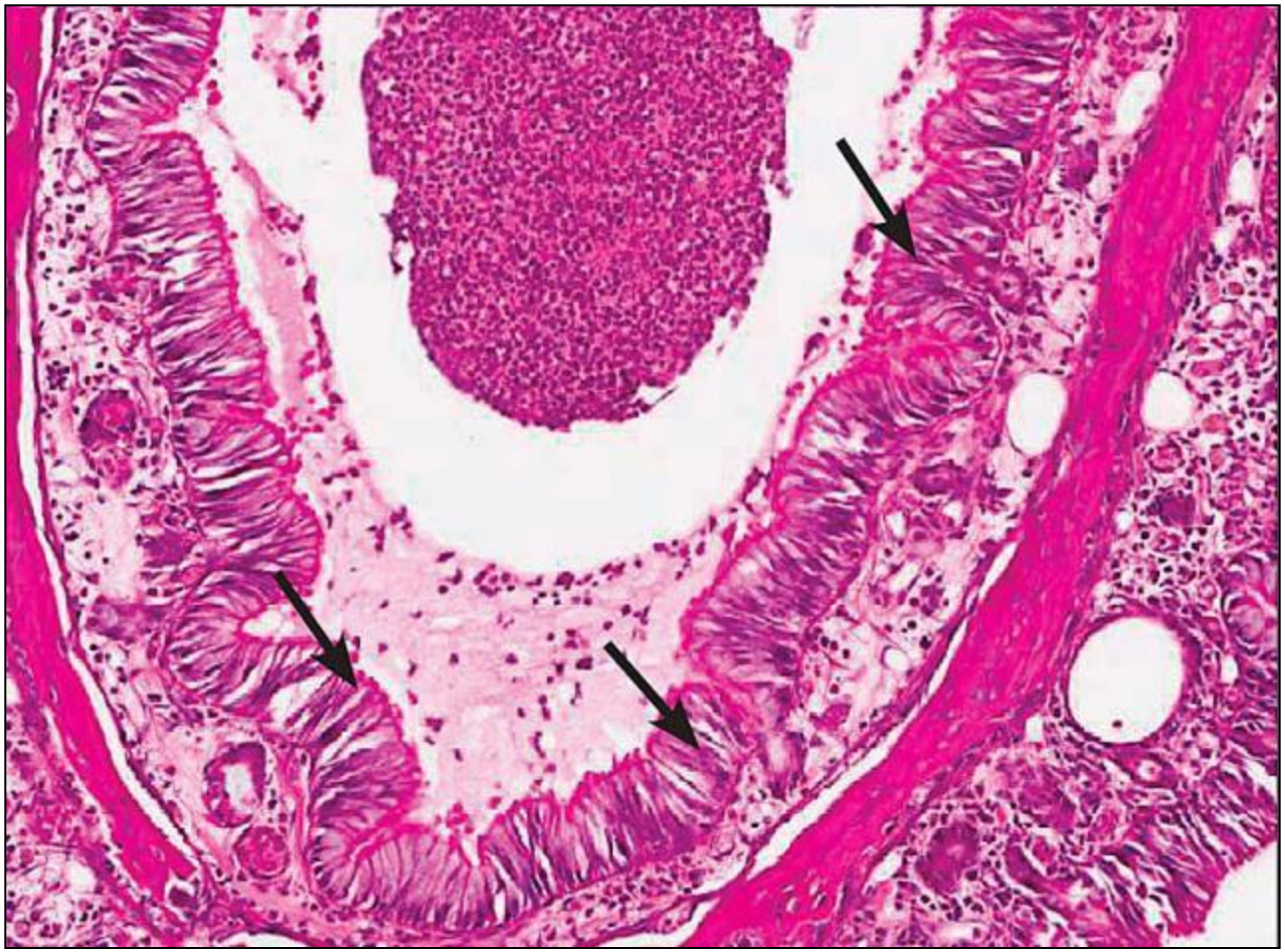
Thyroid Follicular Adenoma in a Male Rat Treated for 2 Years with 1000 mg/kg of Ginkgo Biloba



Thyroid Follicular Carcinoma in a Female Rat Treated for 2 Years with 300 mg/kg of Ginkgo Biloba

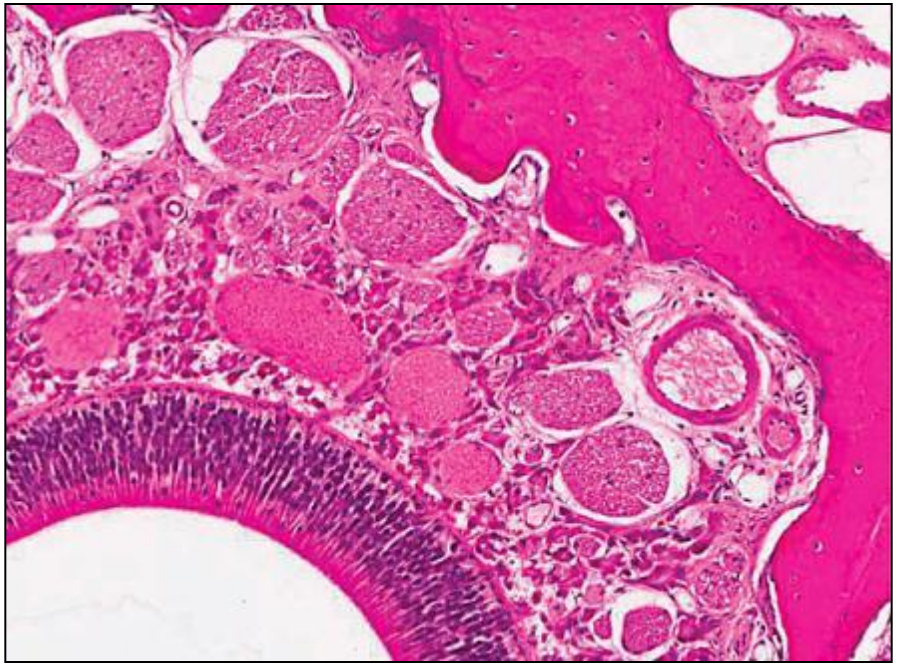


Nose, Level 3: Chronic Active Inflammation and Respiratory Metaplasia of the Olfactory Epithelium in a Female Rat Treated for 2 Years with 1000 mg/kg of Ginkgo Biloba

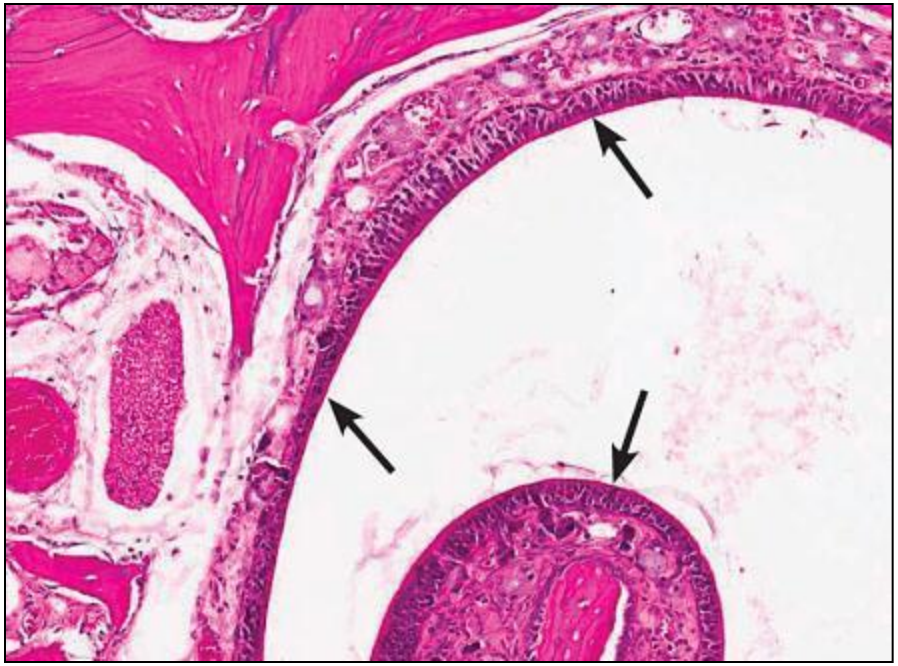


Nose, Level 3: Atrophy of the Olfactory Epithelium in a Female Rat Treated for 2 Years with 1000 mg/kg of Ginkgo Biloba

Control Rat

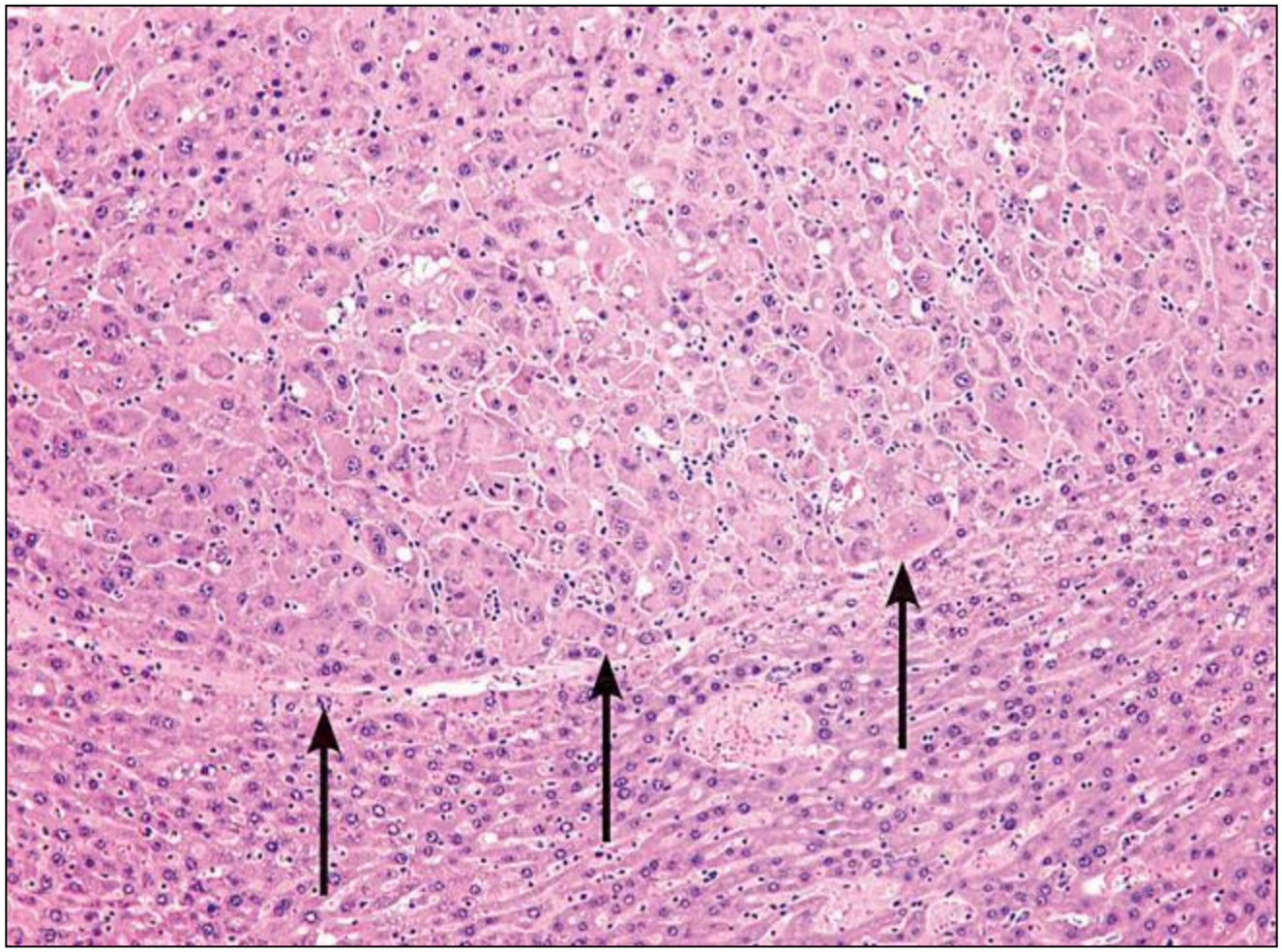


Treated Rat

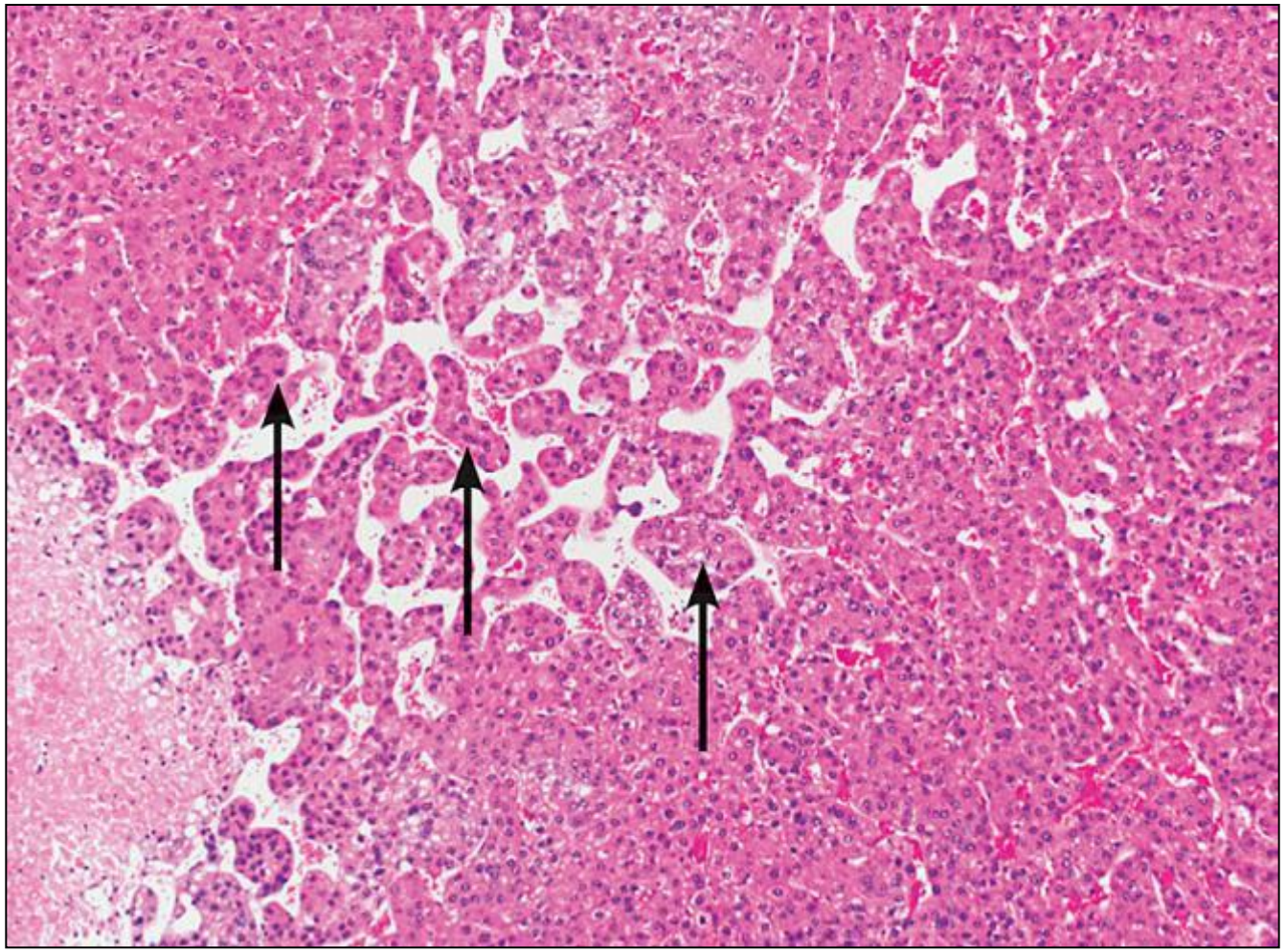


Histopathology Findings
2-Year Studies – Mice

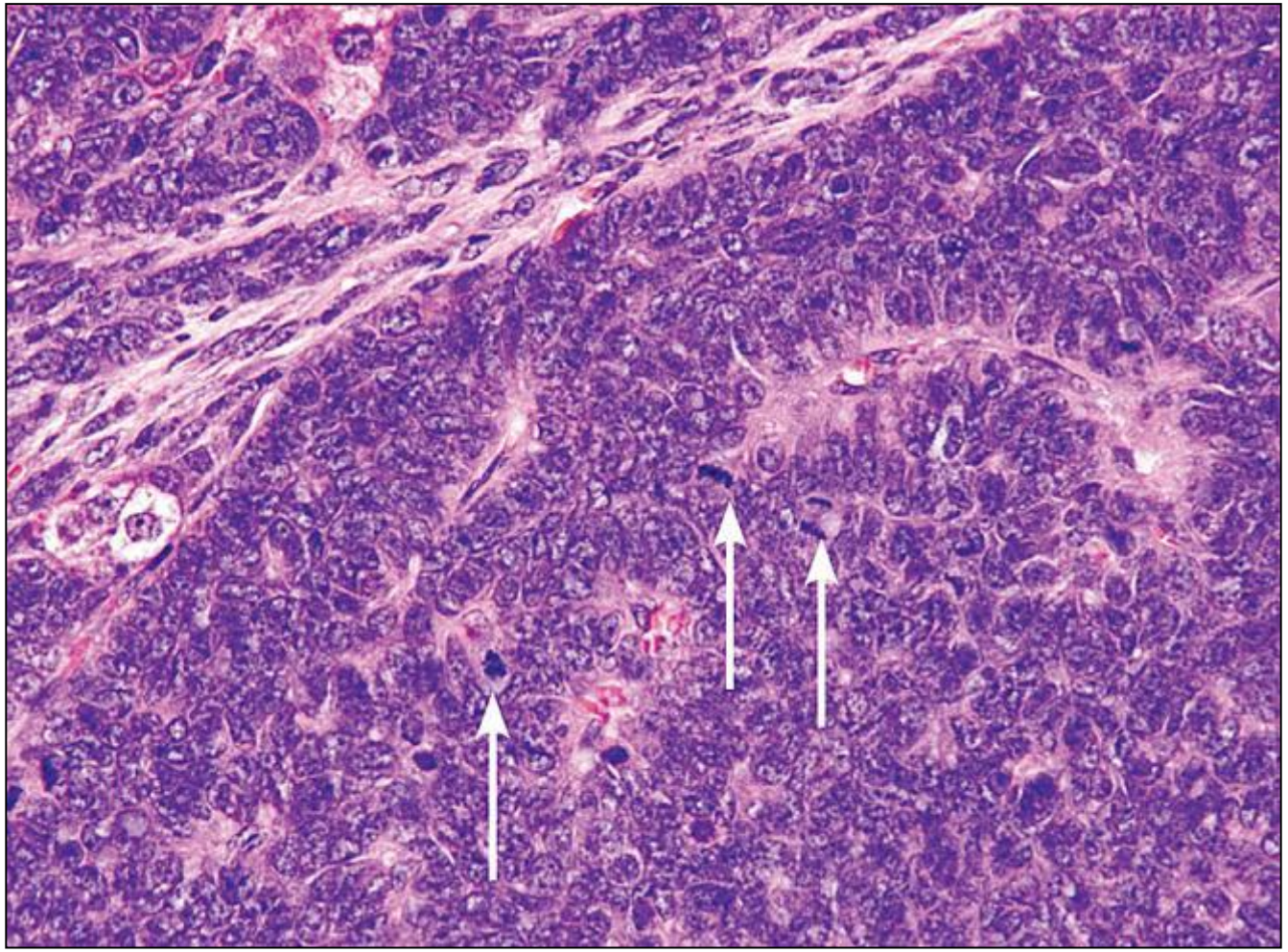
Hepatocellular Adenoma in a Male Mouse Treated with 2000 mg/kg of Ginkgo Biloba for Two Years



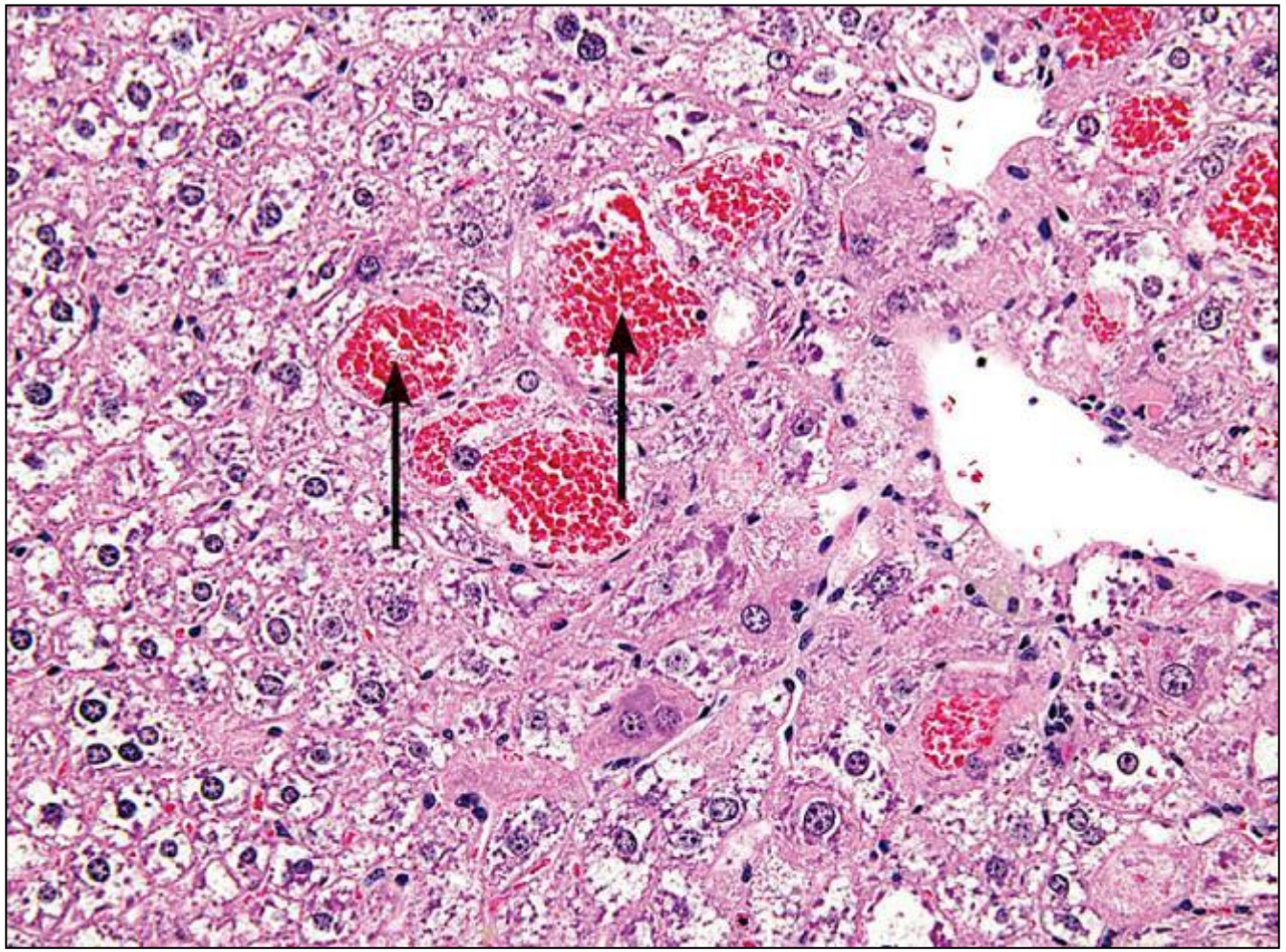
Hepatocellular Carcinoma in a Female Mouse Treated with 2000 mg/kg of Ginkgo Biloba for Two Years



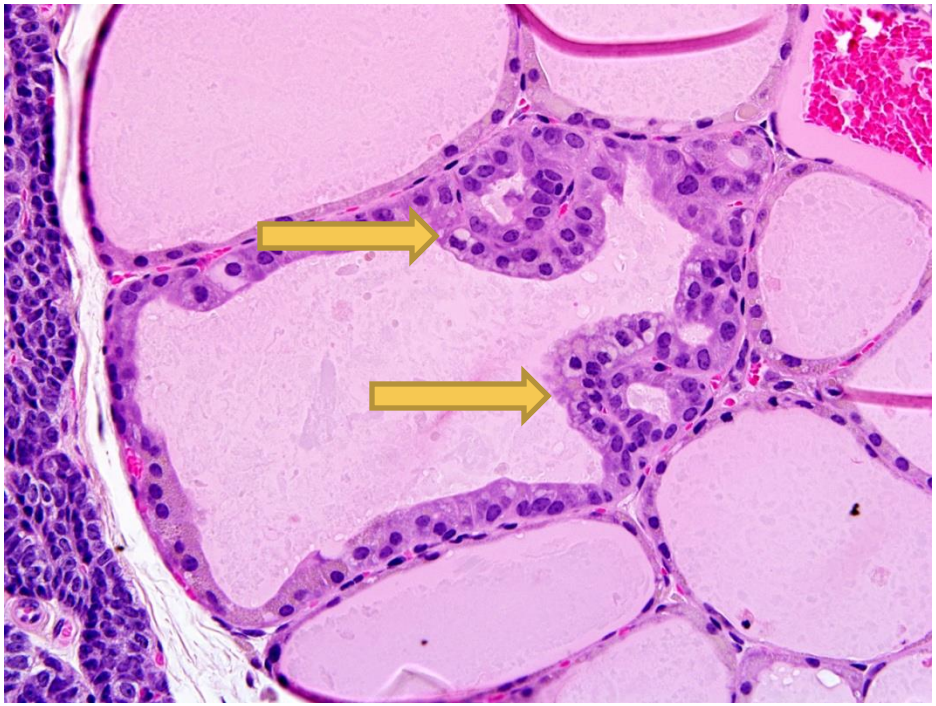
Hepatoblastoma in a Male Mouse Treated with 200 mg/kg of Ginkgo Biloba for Two Years

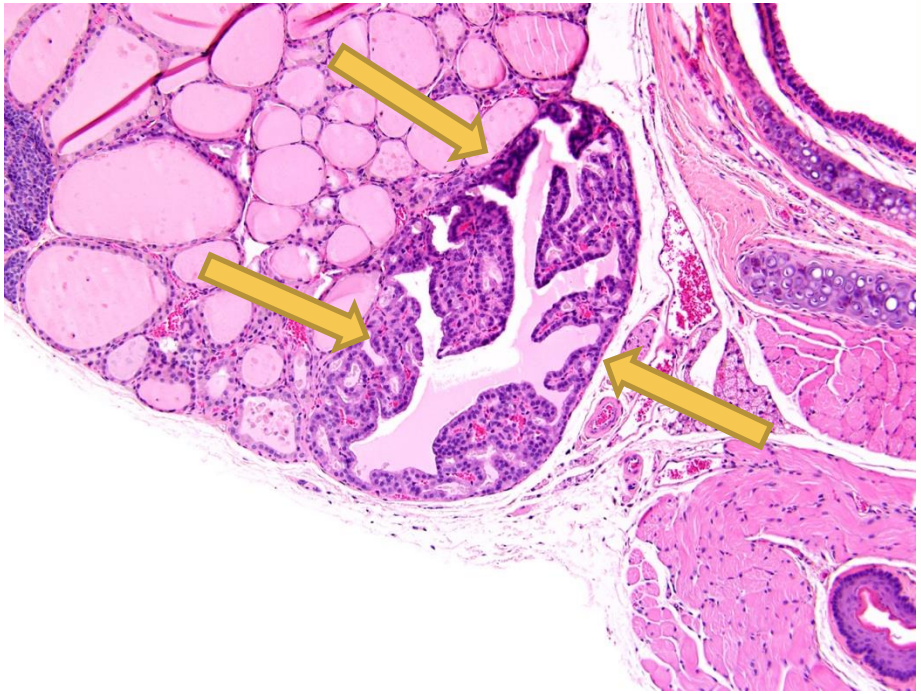


Erythrophagocytosis in a Male Mouse Treated with 200 mg/kg of Ginkgo Biloba for Two Years



Thyroid Follicular cell hyperplasia (left) and follicular cell adenoma (right) in Male Mice Treated with 2000 mg/kg of Ginkgo Biloba for Two Years





NTP
National Toxicology Program

Kava Kava Extract
NTP Technical Report TR 571

Histopathology Findings in 3 Month Study in rats

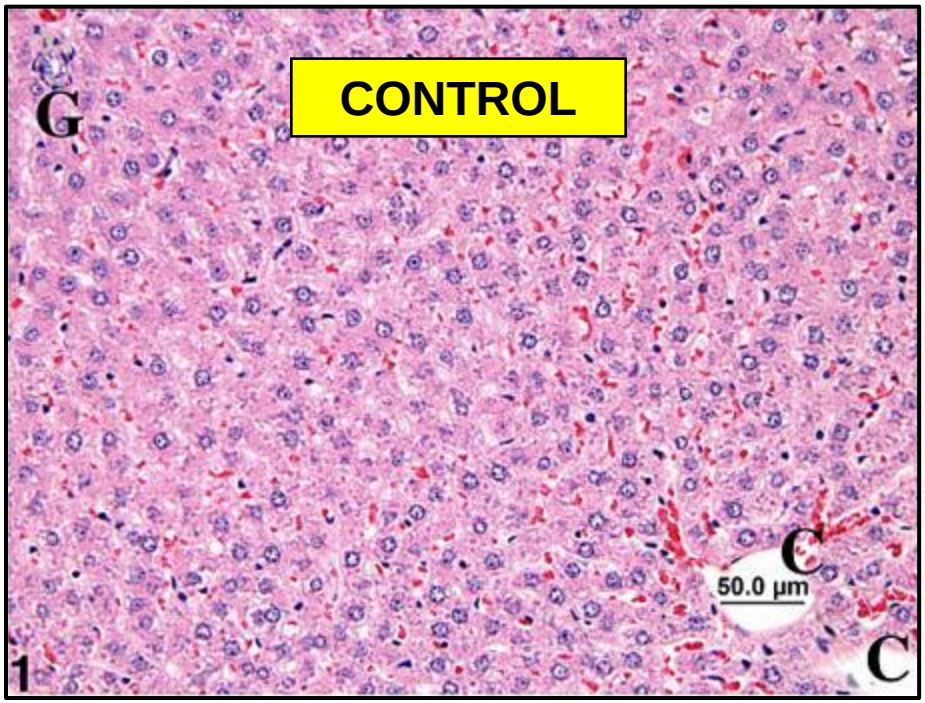
Three-month Study in Rats

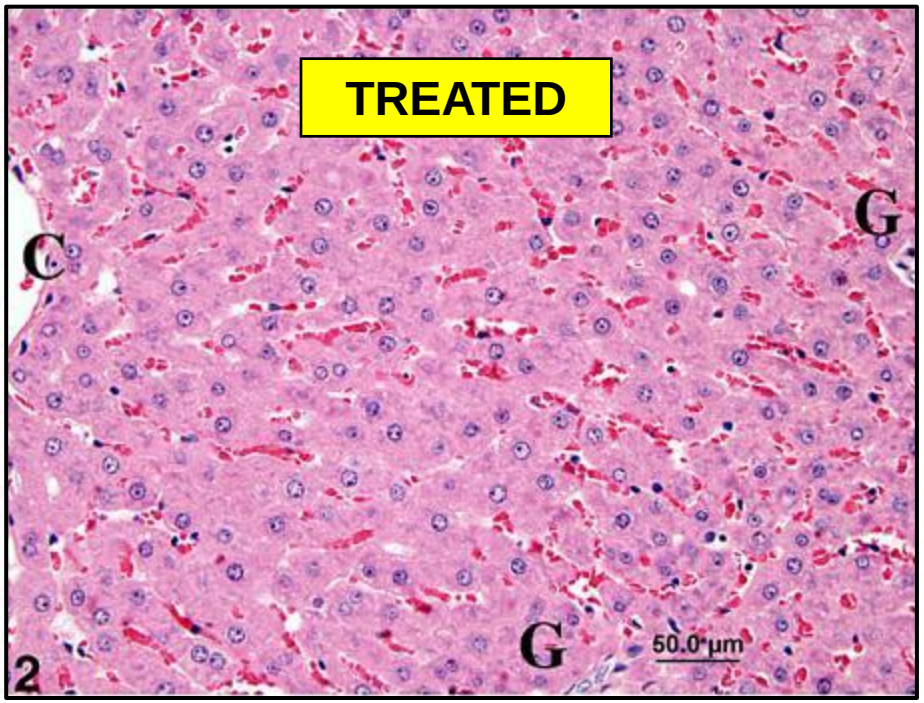
- Increase in liver weights of ≥ 0.25 g/kg males and ≥ 0.5 g/kg females
- Increase in hepatocellular hypertrophy in 2 g/kg females
- Clinical pathology findings considered unremarkable

Immunohistochemical Analysis of CYPs Expression in the Liver Treated with Kava Kava Extract for 3-month in Rats

Fig. 1: Centrilobular area, control female rat. Note relatively smaller size of hepatocytes with cytoplasmic basophilic stippling

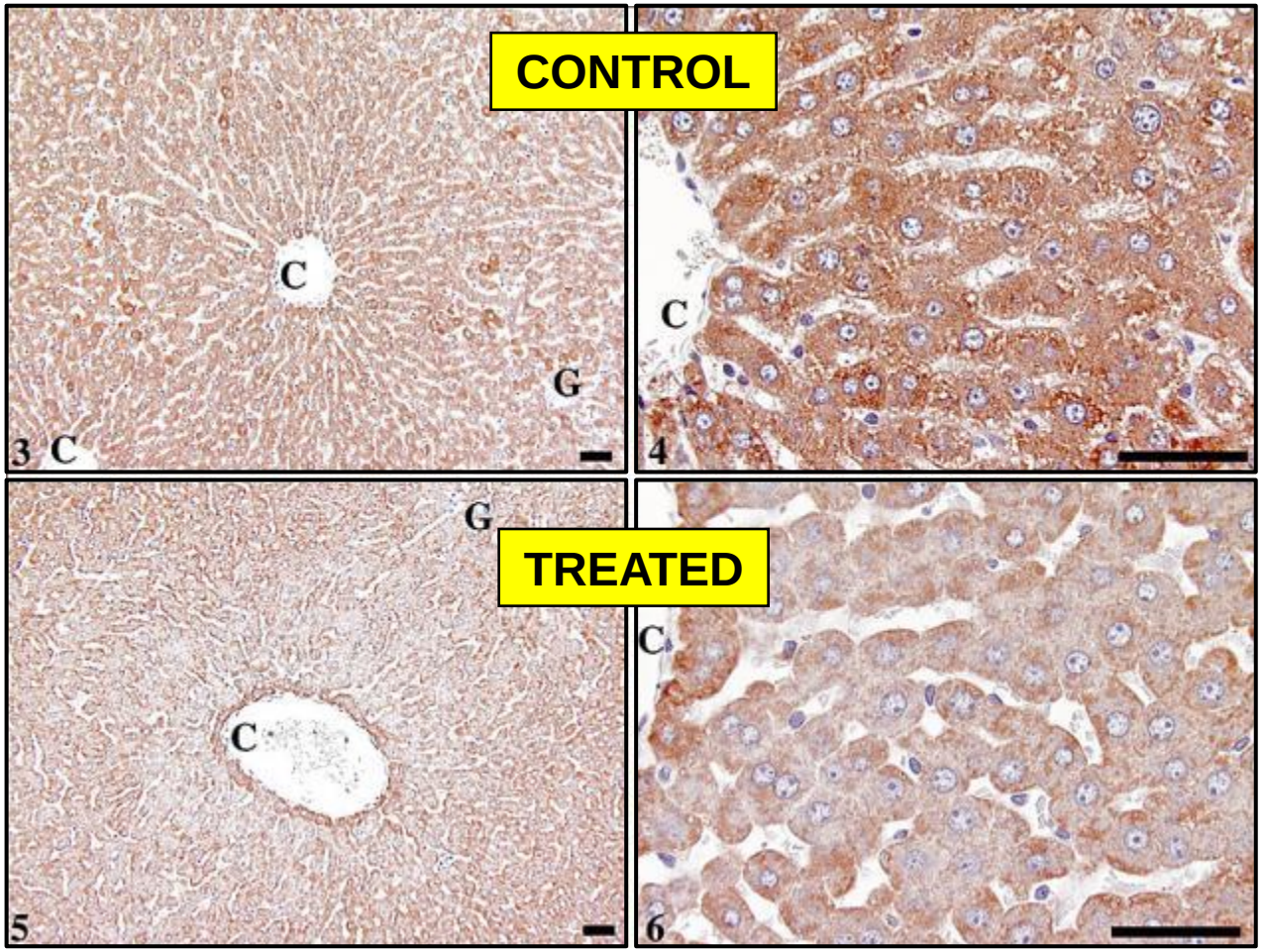
Fig. 2. Mild hepatocytic hypertrophy in female rat treated with 2.0 g/kg kava kava extract. Centrilobular hepatocytes contain more homogeneous eosinophic cytoplasm





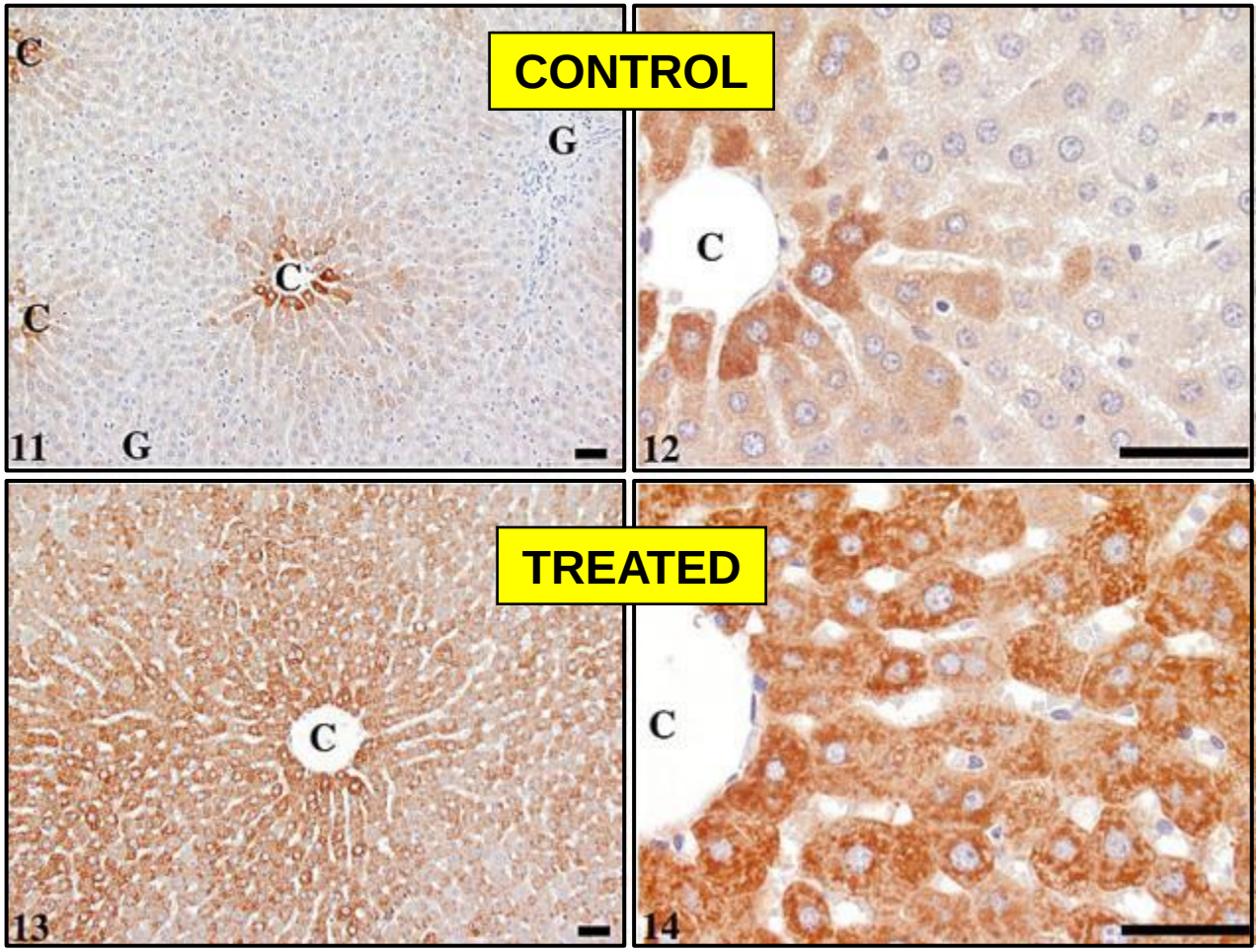
Fig's. 3 & 4:
Strong CYP2D1 expression (intensity: grade 3) in centrilobular area, control female rat; CYP2D1 detected diffusely in cytoplasm of hepatocytes of controls

Fig's. 5&6:
Moderate expression (intensity: grade 2) of CYP2D1 in centrilobular area in female rat treated with 2.0 g/kg kava kava extract by gavage for 3 months



Fig's. 11&12:
Weak expression (relative area: grade 1) of CYP3A1 only in centrilobular area, detected locally in cytoplasm of hepatocytes around central vein, control female rat

Fig's. 13&14:
Strong expression (relative area: grade 4) of CYP3A1 in almost all of centrilobular area in a female rat treated with 2.0 g/kg of kava kava extract by gavage for 3 month



Nondecolorized Whole Leaf Extract of *Aloe Barbadensis* Miller (Aloe Vera) – NTP Technical Report TR 577

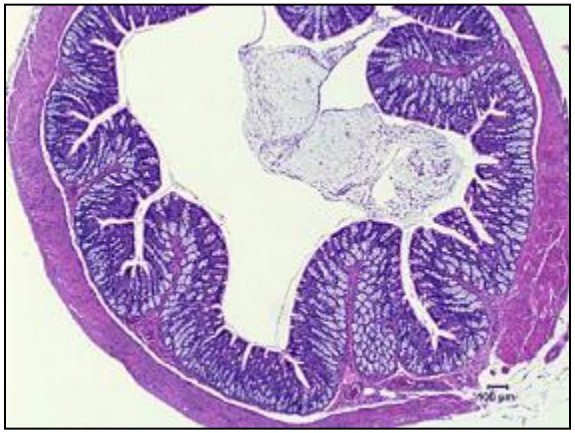
Histopathology Finding

F344/N Rats Administered Aloe Vera Nondecolorized Whole Leaf Extract for 13-Weeks

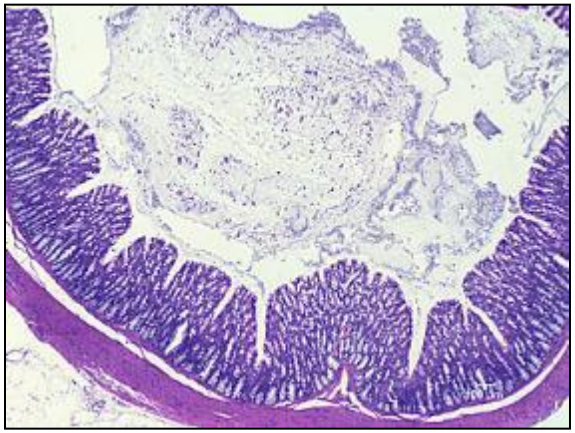
Goblet Cell Hyperplasia seen in the cecum, colon and rectum

The goblet cell hyperplasia may indicate the presence of epithelial cell dysplasia, a precancerous change

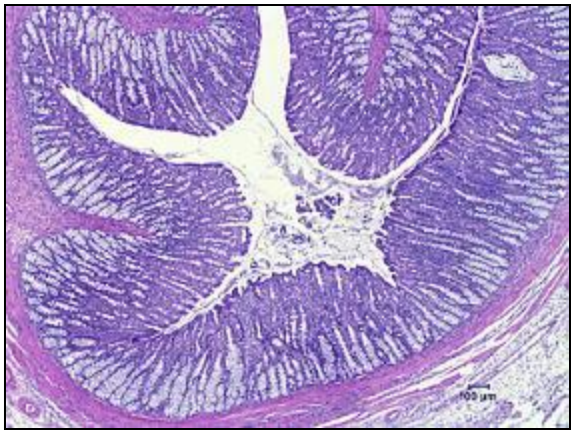
Goblet Cell Hyperplasia in the Colon



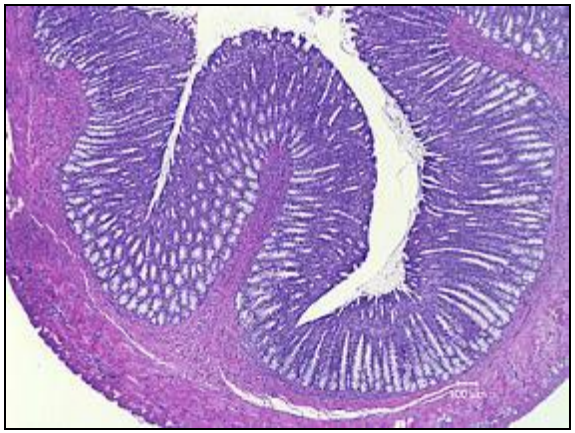
Control 4x



1% Aloe vera whole leaf 4x



2% Aloe vera whole leaf 4x



3% Aloe vera whole leaf 4x

B6C3F1 Mice Administered Aloe Vera Nondecolorized Whole Leaf Extract for 13-Weeks

Goblet Cell Hyperplasia was seen in the cecum, colon and rectum.

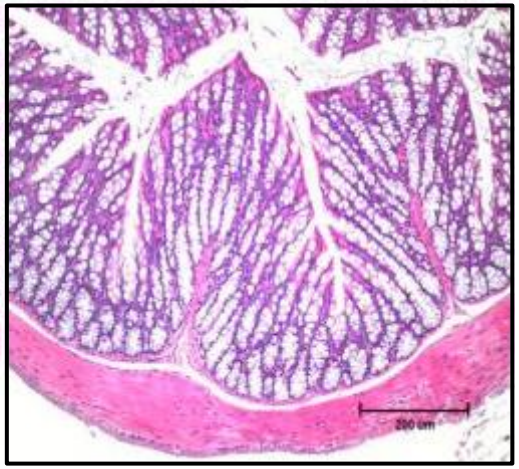
Goblet Cell Hyperplasia in the colon

Control

1% whole leaf extract

2% whole leaf extract

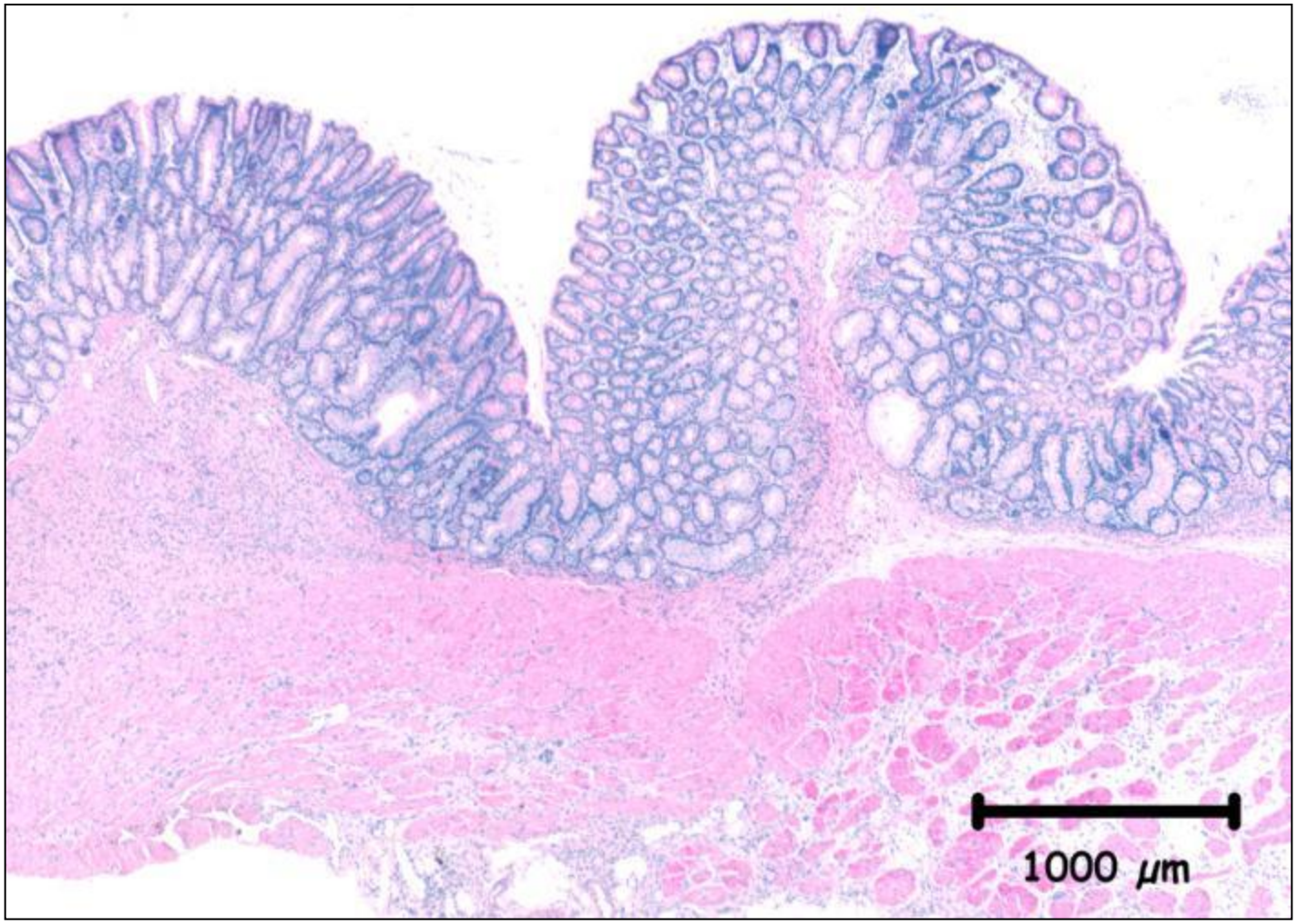
3% whole leaf extract



Lesions of the Gastro-intestinal Tract in F344/N Rats Administered Aloe Vera Nondecolorized Whole Leaf Extract for 2 Years

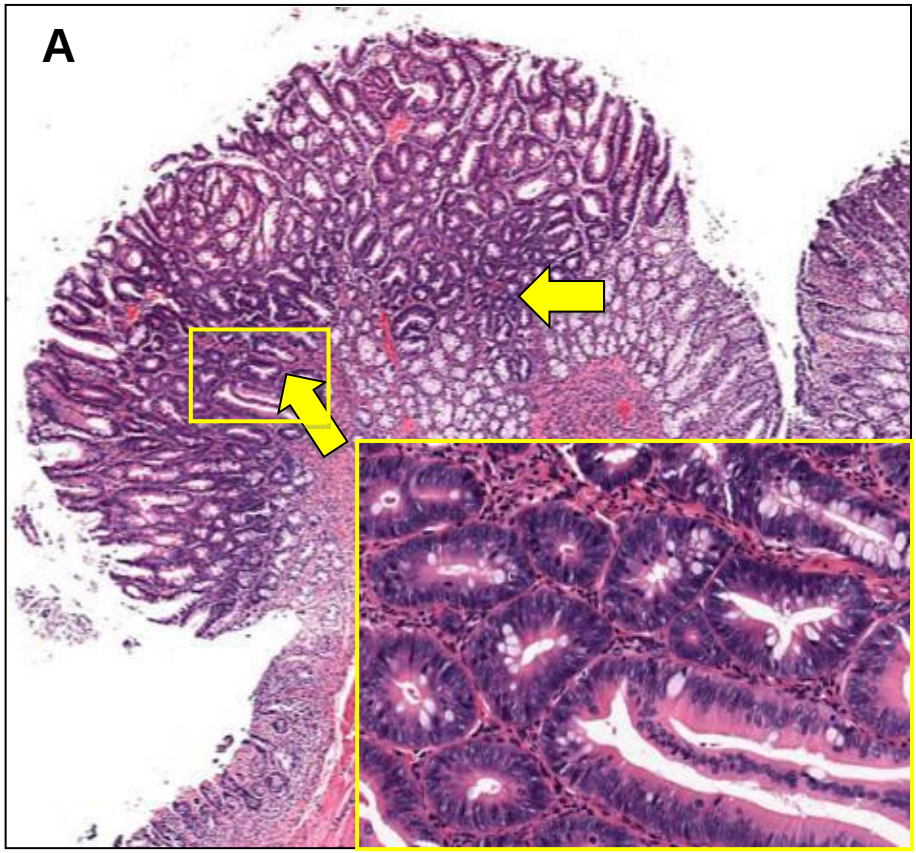
- **Mucosal hyperplasia**
  - Characterized by thickening of the mucosa due to increased length and complexity of mucosal glands, with no cellular atypia and minimal inflammation
  - Dose-related increased incidences in glandular stomach, small intestine, large intestine, and rectum of male and female rats
  - It is uncertain whether the observed changes represent one step in a multistep process of carcinogenesis

Mucosa Hyperplasia of the Large intestinal Tract in F344/N Rats Administered Aloe Vera Nondecolorized Whole Leaf Extract for 2 Years

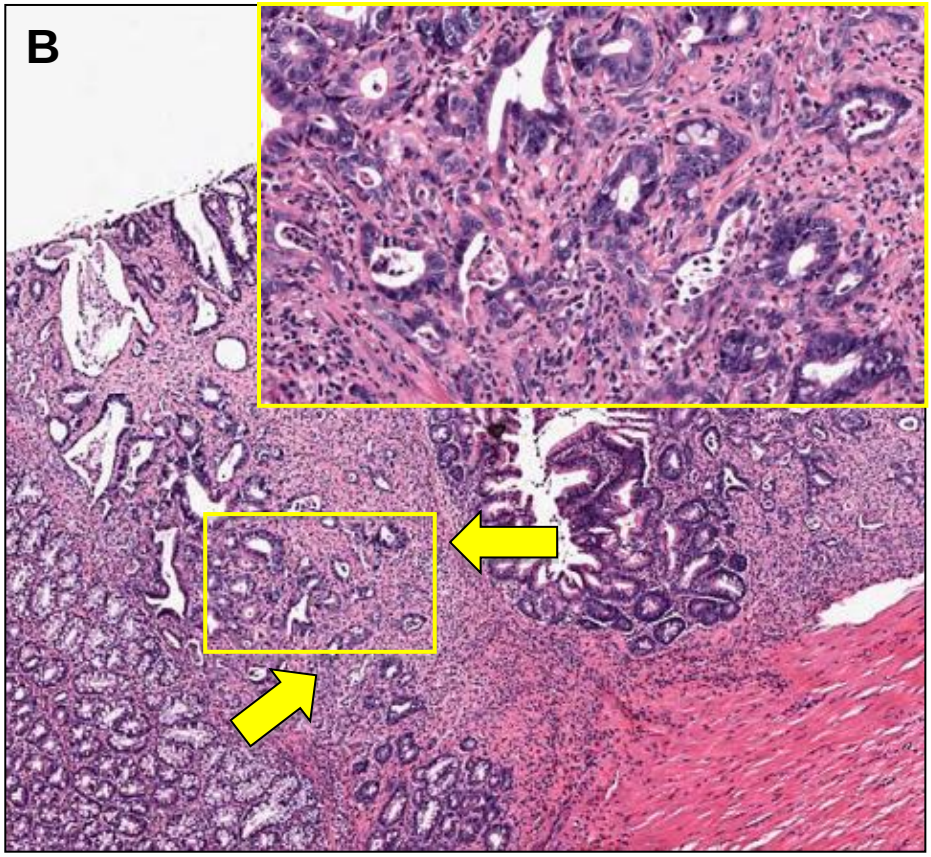


Neoplasms in the Large Intestine of F344/N Rats Administered Aloe Vera Nondecolorized Whole Leaf Extract for 2 Years

- Adenomas – identified as either pedunculated nodules that protruded into lumen or sessile lesions that caused focal thickening of the mucosal wall
- Carcinomas – identified by the invasion of epithelial cells into the stroma of the stalk or into the submucosa and/or muscularis of the intestinal wall



Colon Adenoma

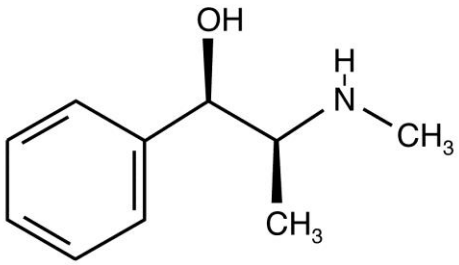


Colon Carcinoma

Ephedrine + Caffeine

Histopathologic Changes in the Heart of Male F344/N Rats

l-Ephedrine hydrochloride

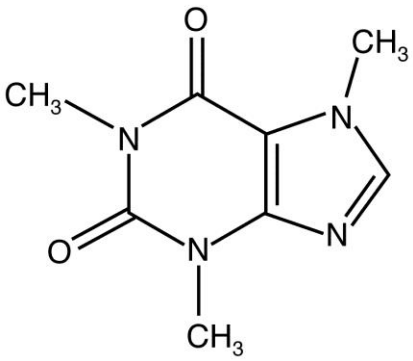


mw (free base) 201.7 (165.2)

$C_{10}H_{15}NO_1HCl$

Cas No. 299-42.3

Caffeine



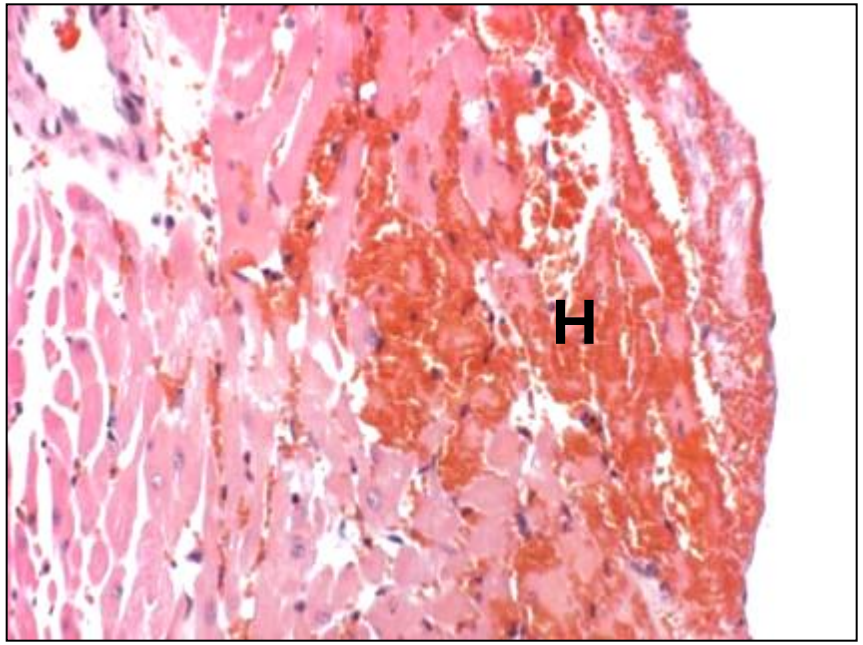
mw 194.19

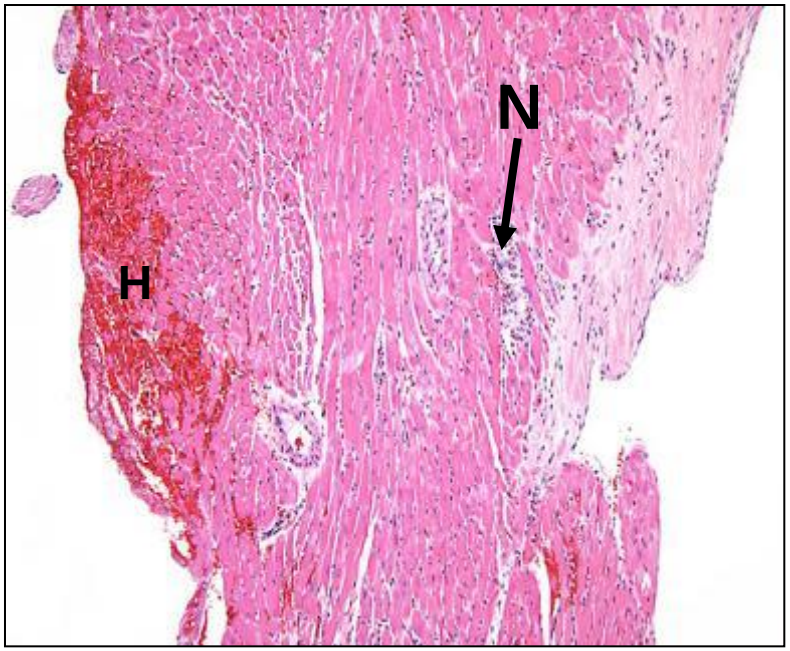
$C_8H_{10}NO_4O_2$

Cas No. 58-08-2

Rat Treated with Ephedrine (25 mg/kg) and Caffeine (30 mg/kg), Died Few Hours After Dosing

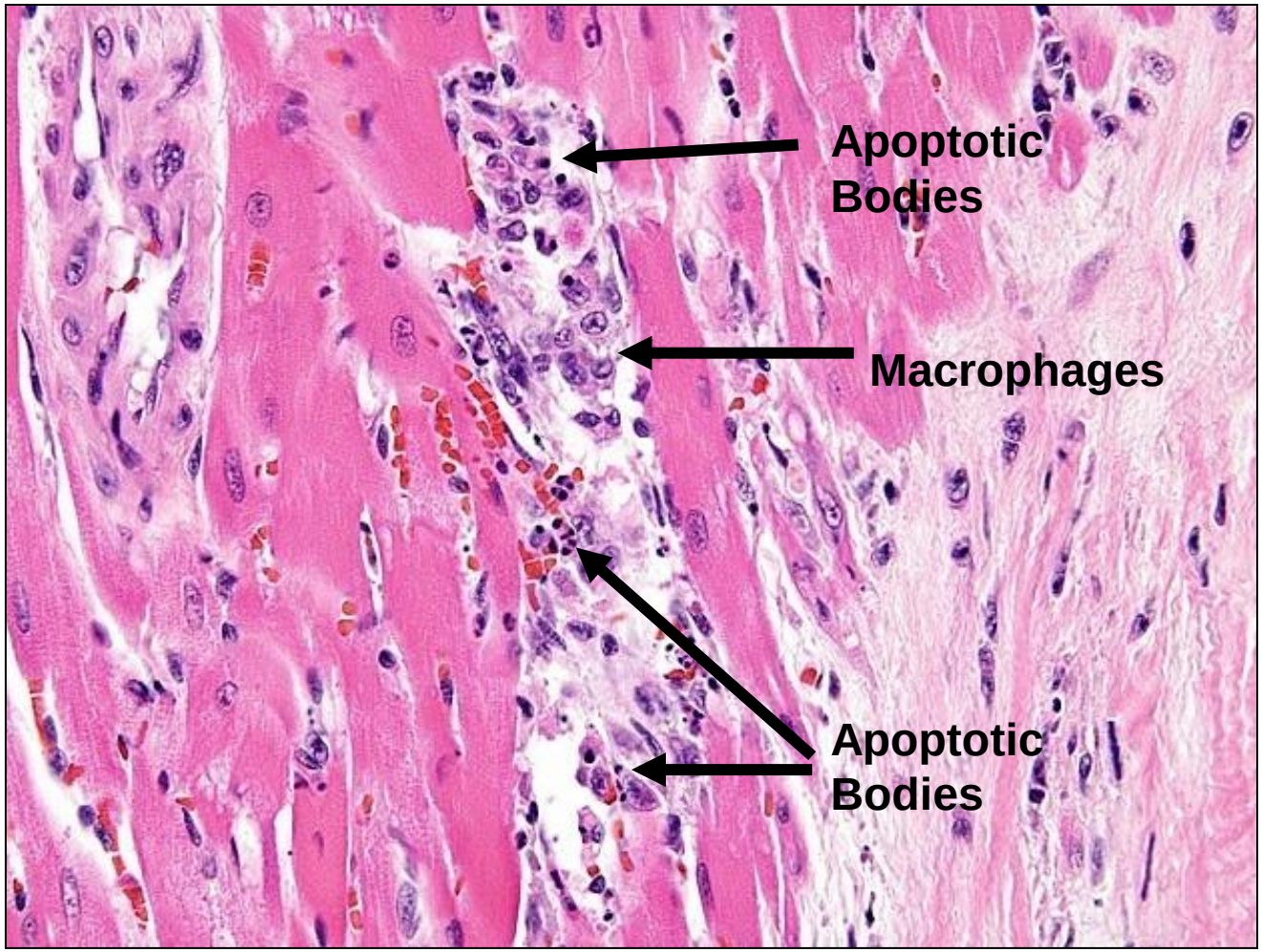
Hemorrhage (H) and Myofiber Necrosis (N) Associated with Macrophages Infiltration in the Left Ventricle





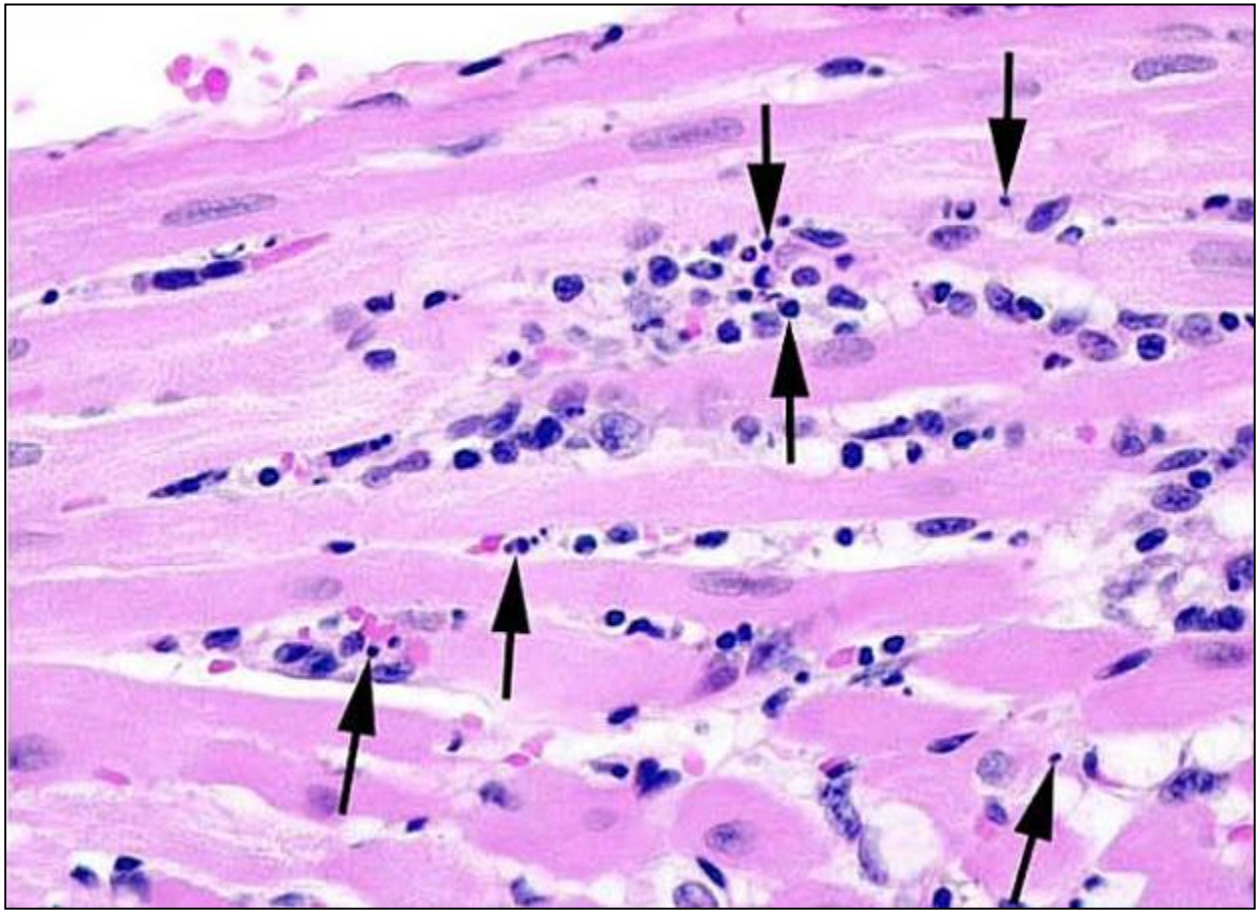
Higher Magnification of the Previous Photo:

Myofiber apoptosis and Macrophages Infiltration



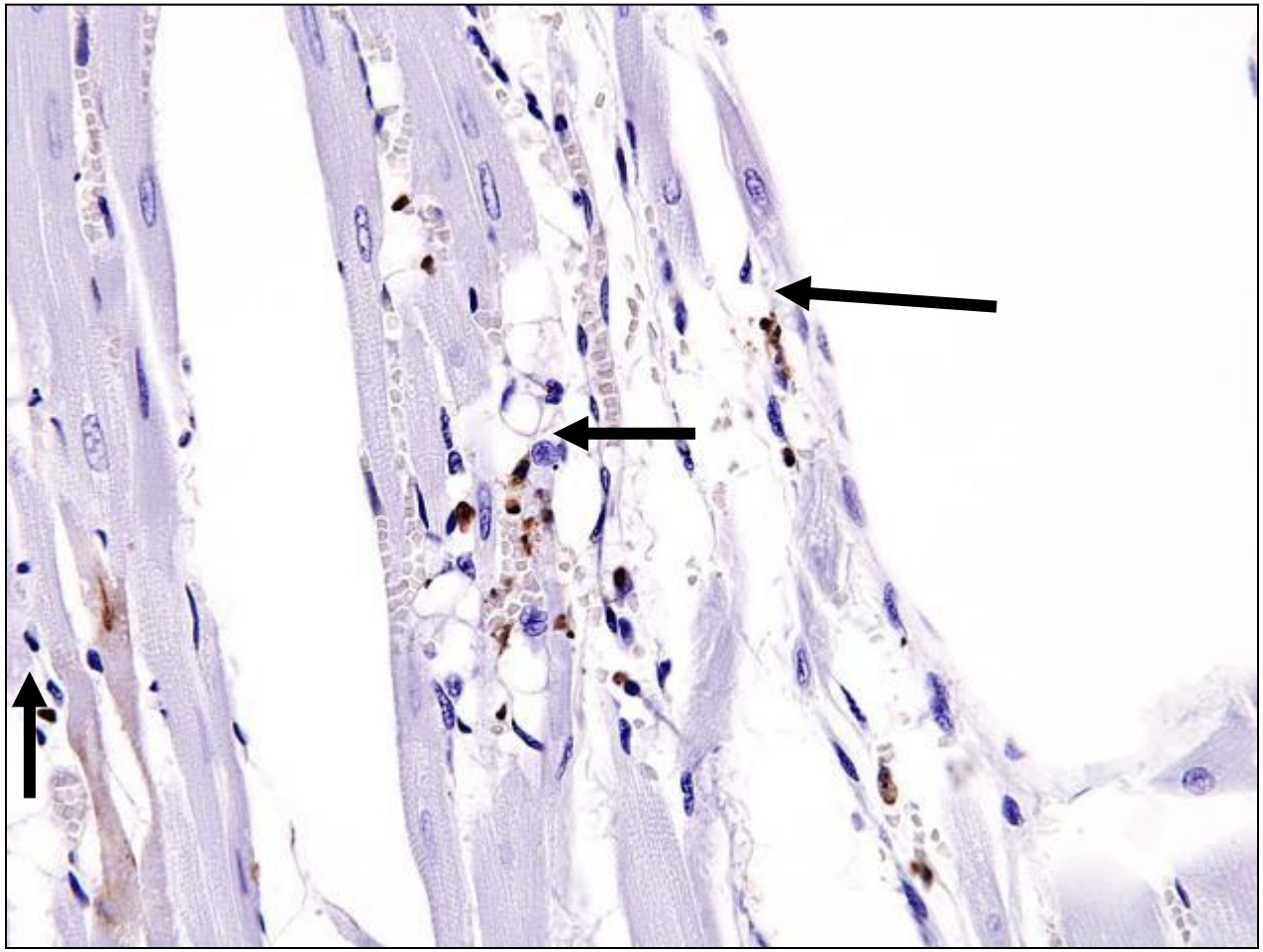
Rat Treated with Ephedrine (25 mg/kg) and Caffeine (30 mg/kg), Sacrificed Animal Few Hours After Dosing

Deeply Basophilic Fragments of Nuclear Debris, Mixed with Some Macrophages



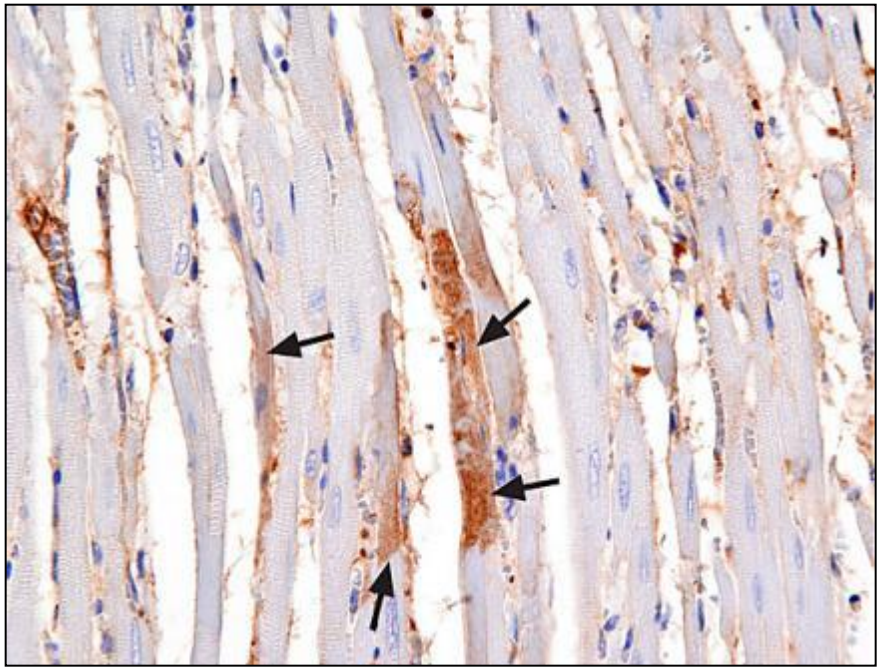
Rat Treated with Ephedrine (25 mg/kg) and Caffeine (30 mg/kg), Died Few Hours After Dosing.

Apoptotic Bodies (TUNEL Staining)

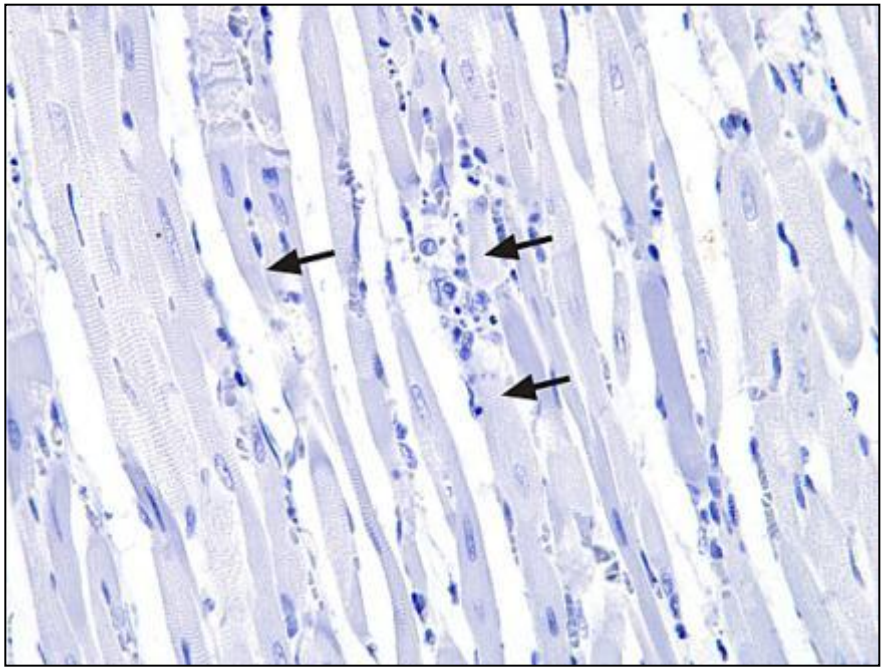


Rat Treated with Ephedrine (25 mg/kg) and Caffeine (30 mg/kg), Died Few Hours After Dosing

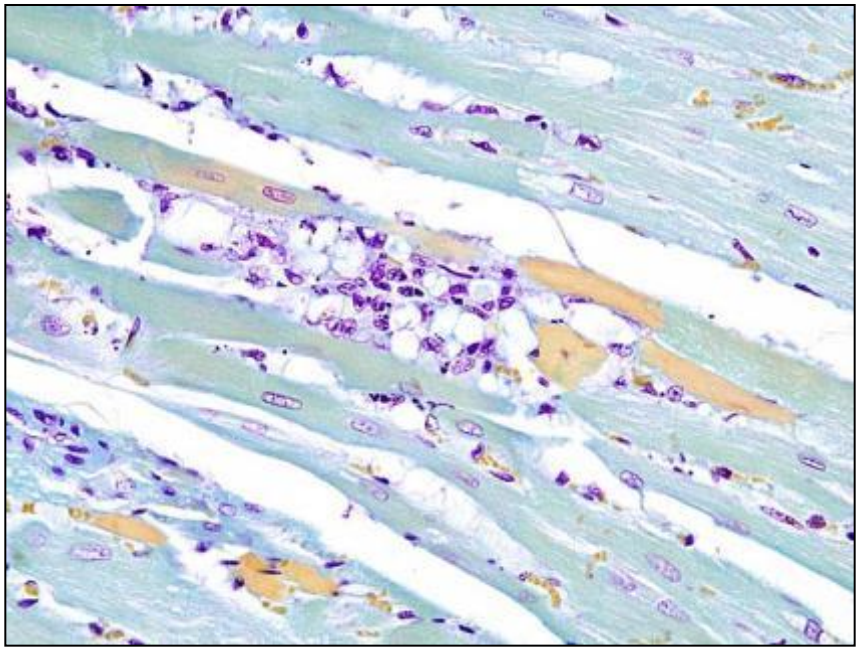
Cleaved Caspase-3 staining



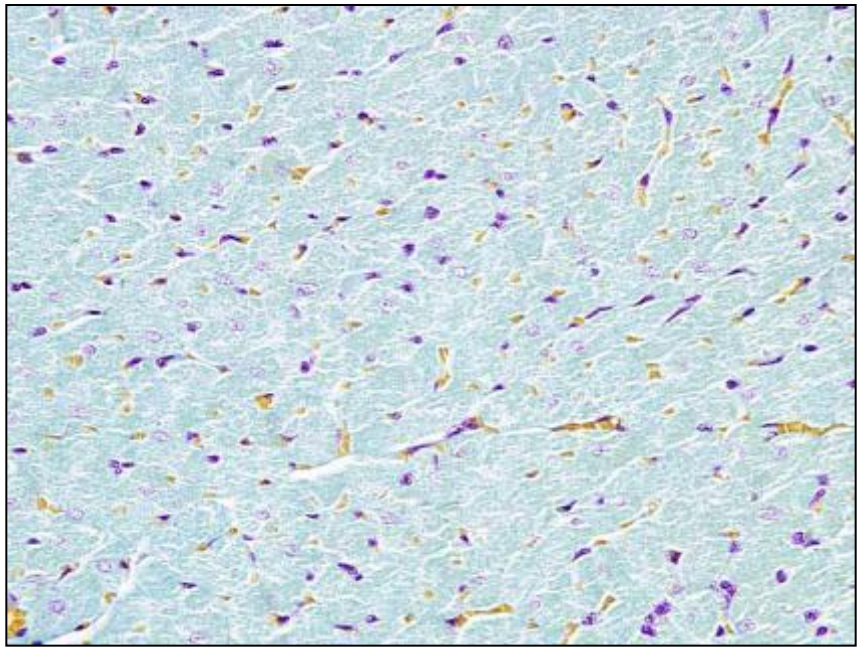
Negative Control (No Antibody for Caspase 3 was Added



Barbeitto-López Trichrome Stain
Myofiber Degeneration and Necrosis



25 mg/kg ephedrine 30 mg/kg caffeine – degenerating and necrotic myofibers are stained yellow



Control rat

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