

APPENDIX

Supplemental Methods

Collection of Urine from Rabbits. An anesthetized rabbit was placed on its back and the lower ventral abdomen was surgically prepped by shaving the fur and applying an antiseptic scrub or solution. The bladder was palpated through the abdominal muscles and gently immobilized against the abdominal wall before needle insertion and aspiration of urine. A small-bore needle (23- to 25-gauge) attached to a 10-mL syringe was then caudally inserted at a 45° angle through the abdominal skin and muscles and into the bladder approximately 3 cm cranial to the junction between the bladder and the urethra. Suction was applied and the urine sample was collected into the syringe.

TABLE 1A

Summary of Blood Chemistry Panel and CBC Comparisons Between Male Rat Groups

Chemistry panel	G1D-1 vs. G1D1	G1D1 vs. G2D1	G3D7 vs. G4D7	G5D14 vs. G6D14
Alkaline Phosphatase (IU/L)	193.0<194.2 (NS)	194.2<292.6 (P<0.01)	210.4>209.4 (NS)	206.8<135.8 (P<0.005)
ALT (SGPT) (IU/L)	63.4<64.6 (NS)	64.6<74.6 (NS)	42.0<48.8 (NS)	161.0>33.6 (NS)
AST (SGOT) (IU/L)	168.8>75.8 (P<0.02)	75.8<94.2 (NS)	77.4<109.6 (NS)	205.0>116.4 (NS)
Creatine Kinase (CK) (IU/L)	1350>281 (P<0.001)	281>175 (NS)	432.2<756.4 (NS)	410.0<828.6 (NS)
γ-Glutamyl Transpeptidase (GGT) (IU/L)	0=0 (NS)	0=0 (NS)	0=0 (NS)	0=0 (NS)
Amylase (IU/L)	977.2<1016.4 (NS)	1016.4>964.2 (NS)	1067>986 (NS)	903.2>819.2 (NS)
Lipase (IU/L)	9.2<22.0 (P<0.005)	22.0>18.8 (NS)	24.2<26.0 (NS)	37.6<39.2 (NS)
Albumin (g/dL)	3.46<3.58 (NS)	3.58>3.56 (NS)	3.84>3.72 (NS)	3.0<3.2 (NS)
Total Protein (g/dL)	7.14<7.58 (NS)	7.58>7.40 (NS)	8.12>7.74 (NS)	6.14<6.46 (NS)
Globulin (g/dL)	3.68<4.00 (NS)	4.00>3.84 (NS)	4.28>4.02 (NS)	3.14<3.24 (NS)
Total Bilirubin (mg/dL)	0.08=0.08 (NS)	0.08<1.00 (NS)	0.02>0.00 (NS)	0.04=0.04 (NS)
Direct Bilirubin (mg/dL)	0.06>0.04 (NS)	0.04<0.06 (NS)	0=0 (NS)	0=0 (NS)
BUN (mg/dL)	15.6=15.6 (NS)	15.6>15.2 (NS)	15.6<16.0 (NS)	18.8>15.0 (P<0.005)
Creatinine (mg/dL)	0.64>0.62 (NS)	0.62>0.60 (NS)	0.72>0.70 (NS)	0.64<0.66 (NS)
Cholesterol (mg/dL)	92.0<104.2 (NS)	104.2>88.2 (NS)	79.8>77.6 (NS)	63<68 (NS)
Glucose (mg/dL)	139<156 (NS)	156.0<160.8 (NS)	107.8<126.4 (NS)	312.6<320.0 (NS)
Calcium (mg/dL)	10.66<11.82 (P<0.001)	11.82>11.64 (NS)	12.18>11.74 (P<0.05)	11.26<11.50 (NS)
Phosphorus (mg/dL)	9.22>9.04 (NS)	9.04>8.84 (NS)	11.28>9.80 (P<0.05)	9.10<9.38 (NS)
Chloride (mEq/L)	97.2<99.6 (P<0.01)	99.6>100.2 (NS)	97.4<98.0 (NS)	99.4>94.8 (P<0.02)
Potassium (mEq/L)	6.56>5.82 (NS)	5.82<5.98 (NS)	7.14>6.68 (NS)	6.00>5.62 (NS)
Sodium (mEq/L)	142.4<148.4 (P<0.001)	148.4=148.4 (NS)	152.8>150.2 (NS)	146.4<146.6 (NS)
Albumin/Globulin	0.94>0.88 (NS)	0.88<0.92 (NS)	0.9=0.9 (NS)	0.94<0.96 (NS)
BUN/Creatinine	24.54<25.24 (NS)	25.24>25.34 (NS)	21.86<22.84 (NS)	29.52>23.00 (P<0.02)
Indirect Bilirubin	0.02<0.04 (NS)	0.04=0.04 (NS)	0.02>0.00 (NS)	0.04=0.04 (NS)
Na/K ratio	22.0<25.6 (P<0.05)	25.6>25.0 (NS)	21.4<22.4 (NS)	24.8<26.8 (NS)

CBC	G1D-1 vs. G1D1	G1D1 vs. G2D1	G3D7 vs. G4D7	G5D14 vs. G6D14
WBC (Thousands/ μ L)	12.04>10.62 (NS)	10.62>9.12 (NS)	10.00<13.22 (NS)	8.58>3.14 (P<0.02)
RBC (Million/ μ L)	8.94>7.68 (P<0.02)	7.68<7.92 (NS)	8.88>8.21 (P<0.01)	7.946>7.532 (NS)
HGB (g/dL)	16.75>14.64 (P<0.05)	14.64<15.42 (P<0.05)	16.28>15.56 (P<0.05)	14.30>14.14 (NS)
HCT (%)	54.5>47.2 (P<0.01)	47.18<48.92 (P<0.05)	53.76>49.36 (P<0.002)	45.84>45.44 (NS)
MCV (fL)	61.0<61.4 (NS)	61.4<61.8 (NS)	60.4=60.4 (NS)	57.8<60.4 (NS)
MCH (pg)	18.70<19.08 (NS)	19.08<19.50 (NS)	18.32<18.96 (NS)	18.04<18.82 (P<0.05)
MCHC (g/dL)	30.70<31.04 (NS)	31.04<31.50 (NS)	30.26<31.52 (P<0.01)	31.26<31.16 (NS)
NRBC (/100 WBC)	0=0 (NS)	0=0 (NS)	0=0 (NS)	0=0 (NS)
Neutrophil seg (%)	7.0<9.8 (NS)	9.8<12.4 (NS)	8.8>7.8 (NS)	13.8<20.0 (P<0.05)
Neutrophil band (%)	0=0 (NS)	0=0 (NS)	0=0 (NS)	0=0 (NS)
Lymphocyte (%)	90.8>87.4 (NS)	87.4>82.4 (NS)	87.6<89.6 (NS)	82.6>74.8 (P<0.005)
Monocyte (%)	1.8<2.6 (NS)	2.6<5.0 (NS)	2.2<2.4 (NS)	2.6<3.6 (NS)
Eosinophil (%)	0.4>0.2 (NS)	0.2=0.2 (NS)	1.2>0.4 (P<0.05)	1.0<1.6 (NS)
Basophil	0=0 (NS)	0=0 (NS)	0=0 (NS)	0=0 (NS)
Platelet estimate*	-0.4<0 (NS)	0=0 (NS)	0=0 (NS)	0=0 (NS)
Polychromasia [†]	1<2 (P<0.005)	2>1 (P<0.001)	1=1 (NS)	1=1 (NS)
Absolute Neutrophil seg (/ μ L)	866.8<973.6 (NS)	973.6<1044.8 (NS)	950.8>892.2 (NS)	1141.8>604.2 (P<0.05)
Absolute Neutrophil band (/ μ L)	0=0 (NS)	0=0 (NS)	0=0 (NS)	0=0 (NS)
Absolute Lymphocyte (/ μ L)	10894>9385 (NS)	9385>7630 (NS)	8701<11917 (NS)	7093.2>2355.2 (P<0.02)
Absolute Monocyte (/ μ L)	215.4<244.6 (NS)	244.6<422.8 (NS)	208.8<385.8 (NS)	247.6>125.4 (NS)
Absolute Eosinophil (/ μ L)	63.4>16.8 (NS)	16.8<22.6 (NS)	118.8>37.8 (NS)	97.4>55.2 (NS)
Absolute Basophil (/ μ L)	0=0 (NS)	0=0	0=0 (NS)	0=0 (NS)

*Less than zero = decreased; greater than zero = increased; zero = adequate.

[†]1 = slight; 2 = moderate.

NS = no significant difference (P > 0.05); seg = segment.

TABLE 2A

Summary of Blood Chemistry Panel and CBC Comparisons Between Female Rat Groups

Chemistry panel	G1D-1 vs. G1D1	G1D1 vs. G2D1	G3D7 vs. G4D7	G5D14 vs. G6D14
Alkaline Phosphatase (IU/L)	132.0<141.4 (NS)	141.4<166.2 (NS)	107.0<113.8 (NS)	127.2>94.8 (P<0.05)
ALT (SGPT) (IU/L)	50.6<57.4 (NS)	57.4<64.4 (NS)	84.8>55.6 (NS)	49.4>47.0 (NS)
AST (SGOT) (IU/L)	113.8>79.8 (NS)	79.8<109.4 (NS)	157.0>91.4 (NS)	95.4>75.2 (P<0.5)
Creatine Kinase (CK) (IU/L)	941.4>199.6 (P<0.005)	199.6>125.0 (NS)	1054.6>253.0 (NS)	708.4>290.2 (P<0.05)
γ -Glutamyl Transpeptidase (GGT) (IU/L)	0=0 (NS)	0=0 (NS)	0=0 (NS)	0=0 (NS)
Amylase (IU/L)	736.6<814.4 (NS)	814.4>798.2 (NS)	946.2>760.4 (P<0.005)	829.4>792.4 (NS)
Lipase (IU/L)	3.8<18.6 (P<0.02)	18.6>10.8 (NS)	9.4<10.6 (NS)	19.4>28.2 (P<0.005)
Albumin (g/dL)	3.96<4.22 (NS)	4.22=4.22 (NS)	4.66>4.10 (P<0.02)	3.98<4.14 (NS)
Total Protein (g/dL)	7.58<8.20 (P<0.02)	8.20>8.08 (NS)	8.74>7.98 (P<0.05)	7.62<7.92 (NS)
Globulin (g/dL)	3.62<3.98 (P<0.05)	3.98>3.86 (NS)	4.08>3.88 (NS)	3.64<3.78 (NS)
Total Bilirubin (mg/dL)	0.06<0.10 (NS)	0.1=0.1 (NS)	0.1=0.1 (NS)	0.1=0.1 (NS)
Direct Bilirubin (mg/dL)	0.02<0.04 (NS)	0.04<0.06 (NS)	0.06=0.06 (NS)	0.06<0.08 (NS)
BUN (mg/dL)	17.8>16.0 (NS)	16.0>15.2 (NS)	19.0>17.4 (NS)	15.2>13.4 (NS)
Creatinine (mg/dL)	0.60<0.68 (P<0.005)	0.68=0.68 (NS)	0.74<0.78 (NS)	0.66>0.64 (NS)
Cholesterol (mg/dL)	92.2<99.0 (NS)	99.0<99.8 (NS)	100.4>103.6 (NS)	93.0<97.6 (NS)
Glucose (mg/dL)	134.8<174.8 (NS)	174.8>165.0 (NS)	160.8<167.8 (NS)	164.8>148.6 (NS)
Calcium (mg/dL)	10.74<11.98 (P<0.002)	11.98>11.90 (NS)	12.36>12.32 (NS)	11.68<12.56 (P<0.05)
Phosphorus (mg/dL)	7.66>7.60 (NS)	7.60>7.34 (NS)	7.96<8.18 (NS)	8.94>8.36 (NS)
Chloride (mEq/L)	98.2<100.6 (P<0.02)	100.6<100.8 (NS)	100.6=100.6 (NS)	99.6>98.6 (NS)
Potassium (mEq/L)	5.10<5.48 (NS)	5.48>5.42 (NS)	5.70>5.56 (NS)	5.24<5.54 (NS)
Sodium (mEq/L)	140.8<147.2 (P<0.001)	147.2<147.6 (NS)	149<150 (NS)	147.8>146.6 (NS)
Albumin/Globulin	1.10>1.04 (NS)	1.04<1.08 (NS)	1.14>1.04 (NS)	1.10>1.08 (NS)
BUN/Creatinine	29.66>23.86 (NS)	23.86>22.54 (NS)	25.86>22.36 (NS)	23.18>20.86 (NS)
Indirect Bilirubin	0.04<0.06 (NS)	0.06>0.04 (NS)	0.04=0.04 (NS)	0.04>0.02 (NS)
Na/K ratio	28.0>27.2 (NS)	27.2>27.4 (NS)	26.2<27.0 (NS)	28.0>26.6 (NS)

CBC	G1D-1 vs. G1D1	G1D1 vs. G2D1	G3D7 vs. G4D7	G5D14 vs. G6D14
WBC (Thousands/ μ L)	12.38>10.98 (NS)	10.98<11.96 (NS)	10.88<11.10 (NS)	9.70>6.76 (NS)
RBC (Million/ μ L)	8.19>7.08 (P<0.01)	7.08<8.03 (P<0.02)	8.28<8.32 (NS)	8.014>7.392 (P<0.05)
HGB (g/dL)	15.72>13.66 (P<0.005)	13.66<15.22 (P<0.05)	15.33<15.42 (NS)	14.88>14.28 (NS)
HCT (%)	49.12>42.76 (P<0.005)	42.76<47.9 (P<0.05)	48.78<49.02 (NS)	46.78>44.70 (NS)
MCV (fL)	60.0<60.4 (NS)	60.4>59.8 (NS)	58.75<59.00 (NS)	58.6<60.6 (NS)
MCH (pg)	19.10<19.32 (NS)	19.32>18.96 (NS)	18.50<18.56 (NS)	18.54<19.32 (NS)
MCHC (g/dL)	31.80<31.98 (NS)	31.98>31.82 (NS)	31.43<31.46 (NS)	31.78<31.96 (NS)
NRBC (/100 WBC)	0=0 (NS)	0=0 (NS)	0.25>0.20 (NS)	0=0 (NS)
Neutrophil seg (%)	10.6>7.2 (NS)	7.2>5.4 (NS)	3.75>3.60 (NS)	6.8<11.0 (NS)
Neutrophil band (%)	0=0 (NS)	0=0 (NS)	0=0 (NS)	0=0 (NS)
Lymphocyte (%)	86.4<88.8 (NS)	88.8<90.6	94.0>93.2 (NS)	88.8>83.4 (NS)
Monocyte (%)	1.6<4.0 (NS)	4.0>3.4 (NS)	2.25<2.40 (NS)	3.4<4.8 (NS)
Eosinophil (%)	1.2>0.0 (P<0.001)	0.0<0.6 (P<0.05)	0.0<0.6 (P<0.05)	1.0>0.8 (NS)
Basophil	0.0<0.2 (NS)	0.2>0.0 (NS)	0=0 (NS)	0=0 (NS)
Platelet estimate	-0.2<0.0* (NS)	0=0 (NS)	0=0 (NS)	0=0 (NS)
Polychromasia	1.0<1.8 [†] (P<0.005)	1.8>1.4 (NS)	1=1 (NS)	1=1 (NS)
Absolute Neutrophil seg (/ μ L)	1225>807 (NS)	807>619 (NS)	386.75>369.00 (NS)	635.6<698.2 (NS)
Absolute Neutrophil band (/ μ L)	0=0 (NS)	0=0 (NS)	0=0 (NS)	0=0 (NS)
Absolute Lymphocyte (/ μ L)	10803>9772 (NS)	9772<10890 (NS)	10280<10344 (NS)	8603>5676 (NS)
Absolute Monocyte (/ μ L)	195.8<401.4 (NS)	401.4<384.4 (NS)	208.25<302.20 (NS)	364.2>323.4 (NS)
Absolute Eosinophil (/ μ L)	139.4>0.0 (P<0.001)	0.0<66.2 (NS)	0.0<63.8 (NS)	97.0>62.4 (NS)
Absolute Basophil (/ μ L)	0=0 (NS)	0=0 (NS)	0=0 (NS)	0=0 (NS)

*Less than zero = decreased; greater than zero = increased; zero = adequate.

[†]1 = slight; 2 = moderate.

NS = no significant difference (P > 0.05); seg = segment.

TABLE 3A
Histopathology of FHBG-Treated and Control Male Rats

Pathological Condition	FHBG					Control				
	27-1	27-2	20-2	30-2	32-2	22	23-1	29-1	29-2	34-1
Multifocal and moderate pancreatic interstitial fibrosis	X									
Chronic, multifocal, and moderate exocrine acinar atrophy with interstitial edema	X									
Chronic, multifocal, and moderate intestinal muscularis hypertrophy in ileum, cecum, and colon			X							
Mild lympholysis	X	X		X	X			X		
Mild-to-moderate, multifocal acute thymic hemorrhage										X
Mild and chronic bile duct hyperplasia							X			X
Mild and focal exocrine pancreas acinar regeneration								X		
Acute and focal mild lingual adenodochitis								X		
Mild hyperplasia of cervical lymph node								X		
Peracute, focally extensive, moderate necrotizing rhinitis in mid nasal turbinates and rhinitis in distal turbinates						X				

Histopathology of FHBG-Treated and Control Female Rats

TABLE 4A

Histopathology of FHBG-Treated and Control Male Rabbits

Pathological Condition	FHBG				Control		
	4104	4283	4289	4413	4103	4285	4290
Acute, multifocal, mild testicular degeneration		X	X				X
Tracheitis					X		X
Thymic hemorrhage			X		X	X	X
Renal tubular mineralization		X	X			X	
Granulomatous bronchopneumonia					X		X
Cervical lymph node lympholysis, hyperplasia and erythrophagocytosis	X						
Pulmonary anthracosis	X						
Cholangiohepatitis	X						
Prostate epithelial hyperplasia	X						
Duodenal submucosal granuloma and enteritis	X						
Adrenal nodular hyperplasia		X					
Peripancreatic hemorrhage		X					
Pulmonary congestion			X				
Spinal cord capillary hemorrhage			X				
Pulmonary edema and congestion				X			
Colonic submucosal edema				X			
Pulmonary nodular calcification						X	
Lymph node congestion and local hemorrhage							X
Pulmonary hemorrhage							X

Histopathology of FHBG-Treated and Control Female Rabbits

Pathological Condition	FHBG			Control		
	4146	4286	4636	4287	4292	4414
Thymic hemorrhage	X	X		X	X	X
Renal tubular mineralization					X	X
Pulmonary congestion	X	X				
Ulcerative tracheitis	X		X	X		

Spinal cord capillary hemorrhage		X			X	
Pulmonary nodular calcification		X				
Ovarian mineralized nodule		X				
Hepatitis			X			
Spinal cord axonal degeneration in thoracic and lumbar region			X			
Lympholysis					X	
Tracheal vascular ectasia and submucosal edema					X	

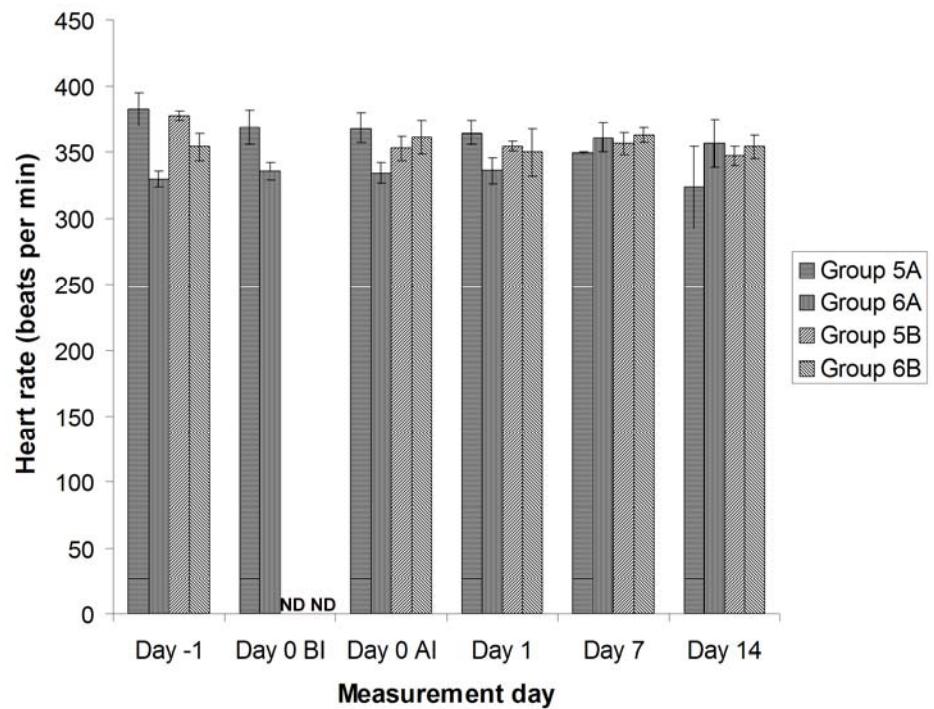


Figure 1A. Heart rate of rats, before and after injection of test articles. Group 5A was control male rats, group 6A was FHBG-treated male rats, group 5B was control female rats, and group 6B was FHBG-treated male rats. $n = 5$ for all rat groups. Values are averages and error bars are SEM. There are significant differences between the average heart rate of FHBG-treated and control male rats on day -1 ($P < 0.005$) and day 0 after injection ($P < 0.05$) but no significant differences on days 0 after injection, 1, 7, and 14. There are no significant differences between the average heart rates of FHBG-treated and control female rats.

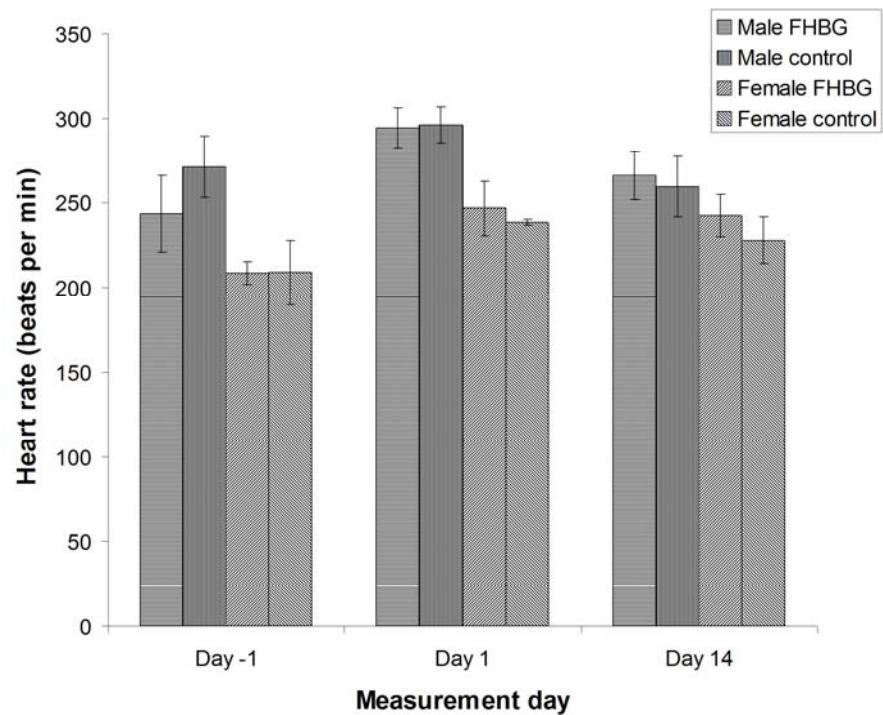


Figure 2A. Heart rates of rabbits as measured by ECG. Data are averages \pm SEM. There are no significant differences between the average values of FHBG-treated vs. control male and female rabbits on any of the measurement days. $n = 4$ for FHBG-treated male rabbits and $n = 3$ for all other groups.