Ministry of Health approved Natural Health Products Testing Agency

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Nutrition and Foods Safety Agency of the Centre for Disease Prevention and Control, People's Republic of China

Testing Report

Sample processing number: 200405018

Name of Sample:	Eleotin
Sample received from:	Botali International Enterprise Co., Ltd Tianjin Port Free Trade Zone
Testing type:	As per requested
Publication date:	January 24, 2004

Nutrition and Foods Safety Agency of the Centre for Disease Prevention and Control, People's Republic of China

Testing Report

Name of Sample:	Eleotin
Sample received from:	Botali International Enterprise Co., Ltd Tianjin Port Free Trade Zone
Manufacturer:	Eastwood Bio-Medical Research Inc.
Sample Number:	20040204
Expiration Date:	24 months from manufacturing date **condition: store under normal room temperature**
Sample Description:	Type: capsule Colour: contents- light brown powder
Amount of Sample Received:	0.3g/capsule x 30 capsules/bottle x 1 bottle + 2500g
Sample Received on:	May 11th, 2004
Testing Criteria:	Acute toxicity test, 3 types of hereditary toxicity tests, 30-day treatment test (animal-testing)
Testing Reference:	Natural Health Products Testing and Evaluation Guidelines- 2003

Testing Results:

Acute toxicity test was performed on female and male rats orally. According to the standards set by Acute Toxicity Classification, Eleotin was determined as non-toxic.

According to the results of the 3 types of genotoxicity assays performed (Ames test, testing for occurrences of mutation of the micronucleus obtained from polychromatic erythrocytes in the bone marrow of mice, and testing for occurrences of sperm mutation in mice), Eleotin was not determined to be a source of genetic mutation.

No abnormality was observed with respect to the active levels and growth of rats after the addition of Eleotin to their normal diet at 0.75, 1.50, and 3.00g/ kg.BW. Testing of biochemical indexes in blood serum: male 0.75g/ kg.BW treatment group produced cholesterol levels higher than that of the control group, male 1.50 and 3.00g/kg.BW treatment groups produced ALB (Albumin) levels lower than that of the control group ($p<0.05$), references with control group levels from past experimental records within this laboratory); except for the differences described above, all other biochemical indexes of treatment groups (both male and female) are similar to those of the control group. Eleotin was also observed to not have any negative effects on the following: weight of subjects, digestive efficiency & food usage, haematology indexes, mass of internal organs, mass of internal organs to body mass ratio, and pathological histology. (end of page)

Eleotin: Toxicology Report

1 Materials and Methods

1.1 Sample: Eleotin, provided by Botali International Enterprise Co., Ltd, Tianjin Port Free Trade Zone. Contents of the capsules are light brown in powder form. The recommended dose for adults is 6 capsules a day at 0.3g/capsule (1.8g/ day), an equivalent of 0.03g/kg.BW (Calculations based on an adult body weight of 60kg).

1.2 Subjects: Healthy Kunming mice supplied by the Chinese Academy of Medical Science, School of Research Animals' Breeding Centre, license #: SCXK (Beijing) 2000-0006. B-grade SD rats provided by Beijing City, Centre for Research Animals, license #: SCXK (Beijing) 2002-0003.

1.3 Instrument and Testing Reagent: Beckman GS15R centrifuge; Hitachi 7060 automatic biochemical analyzer; BECKMAN COULTER® A^{c}·T diff2™ Haematology Analyzer; Nikon electron microscope.

1.4 Experimental Procedure:

1.4.1 Acute toxicity test (subjects: rats): maximum tolerated dose (MTD) test. Twenty healthy SD rats (10 male and 10 female) weighing 180-220g were selected for testing. Subjects were each given 10.0g/kg.BW of Eleotin at 20ml/kg.BW daily for 14 days, during which, subjects were observed for symptoms of poisoning and cases of death.

1.4.2 Genotoxicity assays:

1.4.2.1 Ames test: Approved tester strains, TA97, TA98, TA100, and TA102 were combined with S-9 mix, Eleotin sample, a trace of histidine, and molten agar in Salmonella typhimurium reverse mutation assay. The test was activated by S-9 mix obtained from PCB-induced rat liver preparations. According to the results of the toxicity test, doses of 0.008, 0.04, 0.02, 1.00, and 5.00 mg/dish were determined. Eleotin was dissolved in sterile water, with the largest dose being 1g of Eleotin/20ml of sterile

water; other doses were prepared by diluting doses of Eleotin in sterile water, each dose with an Eleotin increase of 5 times the amount of the previous dose, with 100 μl of prepared sample added to each dish. Unprocessed-control, solvent control, and positive control groups were also established. Tester strains were added at 0.1 ml/strain to agar dishes. Bacterial growth-promoting solution (a trace of histidine) was added to the surface of the agar in each dish, at 0.1 ml/dish. Prepared Eleotin dose samples were also added at 0.1ml/dish, along with 0.5ml S-9 mix/dish (when activation was necessary). Dishes were then left alone for growth in 37°C for 48 hours, after which revertant colonies were counted for each dish. When the number of revertant colonies of the Eleotin dose samples are at least twice more than that of the solvent control group, and with dose-specific differences, the testing result are considered positive. The entire test was repeated a second time under similar conditions.

1.4.2.2 Test of occurrences of mutation of the micronucleus obtained from polychromatic erythrocytes in the bone marrow of mice: Eleotin solutions (or cyclophosphamide, or distilled water) were orally fed to the mice twice at t = 0h and t = 24h. Fifty mice weighing 25-30g were selected and randomly divided into 5 groups of 10 (5 male and 5 female) according to their relative weights. The positive-control group was given 40 mg/kg.BW of cyclophosphamide, the baseline-control group was given distilled water, Eleotin groups were given processed Eleotin (Eleotin powder diluted with distilled water) at doses of 0.83, 2.50, and 7.50 g/kg.BW (high dose meaning maximum dose given at once). At t = 30h, subjects were severed by dislocating the cervical vertebrae. Bone marrow was extracted from bones in the thoracic region (ribs), diluted with veal blood serum, smeared onto glass slides, stabilized with methanol, and finally stained with Giesma. Slides were studied under the electron microscope; the number of polychromatic erythrocytes (PCE) in 200 red blood cells was counted per subject with its percentage calculated. The number of mutated micronuclei in 1000 polychromatic erythrocytes was counted per subject; (‰) occurrence of mutation of the micronucleus was calculated. Statistical analysis was performed on all data.

1.4.2.3 Testing for occurrences of sperm mutation in mice: Twenty-five sexually mature male mice weighing 25-30g were selected and randomly divided into 5 groups of 5. The positive control group was given 40 mg/kg.BW of cyclophosphamide, the baseline-control group was given distilled water, Eleotin groups were given processed Eleotin (Eleotin powder diluted with distilled water) at doses of 0.83, 2.50, and 7.50 g/kg.BW (high dose meaning maximum dose given at once). Respective treatments were orally fed to the subjects (Eleotin solution, cyclophosphamide, or distilled water) once a day for 5 days. Thirty days post treatment, subjects were severed by dislocating the cervical vertebrae. Both testes were removed from subjects, fat in the testes was removed, and the testes were cut into pieces in normal saline, centrifuged at 1000 rev./min for 7 minutes, removed the upper clear layer, smeared the remaining sample onto glass slides, stabilized with methanol, and finally stained with 1.5 % Giesma. Slides were studied under the electron microscope; 1000 sperms were counted per subject, the percentage of mutated sperms was calculated. Statistical analysis was performed on all data.

1.4.3 Thirty-Day Eleotin intake test: 80 weaned rats weighing 56-79g each, randomly divided into 4 groups, 1 control group and 3 experimental groups (at doses of 0.75, 1.50, 3.00g/kg.BW, equivalent to 25, 50, and 100 times that of the recommended dose for human). 10% Eleotin was added to the normal diet of the treatment groups, at 0.75, 1.50, and 3.00% respectively (of total food intake). Subjects were caged and fed separately with free access to water and food. Weights were measured on a weekly basis, and amount of food intake recorded. Subjects were observed for 30 days. At the end of this period, blood samples were obtained after fasting, haematology indexes and blood serum biochemical indexes were tested.

1.4.3.1 Parameter / Index of Observation:

1.4.3.1.1 Behaviour, weight, digestive efficiency & food usage of subjects.

1.4.3.1.2 Haematology and blood serum biochemical indexes: the following were counted: leukocytes, erythrocytes, haemoglobin, AST, ALT, urea (BUN), Cre, TC, TG, blood glucose level, TP, ALB.

1.4.3.1.3 Mass of internal organs to body mass ratio of subjects: At the end of testing, subjects were put down. Liver, kidneys, spleen, and testes were obtained and used in the calculation of the ratio of mass of internal organs to body mass (unit: g).

1.4.3.1.4 Pathological histology: Anatomical dissection and pathological histology testing. When no obvious abnormality was observed with respect to mutation/ cancer and biochemical indexes across all Eleotin dose groups, the high dose group was then more closely examined for possible pathology in the liver, kidneys, testes (or ovaries), stomach, duodenum, and spleen.

1.4.3.2 Statistical analysis of data: mean values and standard deviations were calculated with Excel, variance analysis was performed with PEMS, appropriate $\log(Y_i)$ transformation was performed on means with non-normal distributions and/or inequality of variance to stabilize the variance.

2 Results:

2.1 Acute toxicity test (rats): Table 1 shows that subjects (male and female) that were orally fed Eleotin at 10.0g/kg.BW for 14 days did not suffer from any signs of food poisoning nor death. Results showed that the maximum tolerated dose of Eleotin for SD rats (male and female) was greater than 10.0g/kg.BW with a LD_{50} of greater than 10.0g/kg.BW as well. According to Acute Toxicity Classification, Eleotin was determined as non-toxic.

Table 1. Results of acute toxicity test on subjects (rats) after taking Eleotin for 14 days

Subjects	Sex	Method	LD_{50} (g/kg.BW)
Rats	Female	Orally	>10.0
	Male	Orally	>10.0

2.2 Genotoxicity assays:

2.2.1 Ames test: Tables 2 and 3 show that the number of revertant colonies of the control group fall within the normal range, while the number of revertant colonies for all doses of Eleotin groups do not exceed those of the solvent-control group by more than 2 times, hence no dose-specific differences were observed. Results of the Salmonella typhimurium reverse assay (TA97, TA98, TA100, TA102, with or without S-9) determined that Eleotin does not cause genetic mutation.

Table 2. Results of Eleotin Ames test: 1

Dose (mg/dish)		TA97		TA98		TA100		TA102	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
E	0.008	158.7	139.0	41.7	40.3	148.0	147.7	301.7	292.7
L		±15.3	±11.0	±10.8	±8.4	±11.5	±22.8	±19.1	±4.9
E	0.04	150.0	151.3	49.3	38.0	141.7	144.3	294.0	284.0
O		±9.6	±12.0	±9.5	±4.6	±21.1	±20.6	±1.0	±10.0
T	0.20	172.3	159.3	35.7	43.3	156.7	139.0	284.3	281.0
I		±20.0	±6.4	±9.5	±9.5	±16.0	±18.7	±19.0	±30.5
N	1.00	156.7	148.0	37.7	35.3	140.3	169.3	290.0	313.0
		±9.2	±10.4	±7.5	±4.0	±22.2	±16.2	±9.5	±30.5
	5.00	159.3	160.7	40.7	47.3	151.3	142.0	274.7	294.0
		±11.0	±21.5	±4.5	±14.6	±26.1	±15.1	±11.0	±3.5
Unprocessed-control		153.3	149.0	42.3	42.3	134.7	151.7	294.7	302.3
		±14.2	±13.9	±9.5	±3.1	±9.5	±14.3	±20.6	±13.4
Solvent-control		151.7	151.0	40.3	38.7	±140.7	150.3	301.0	284.3
		±17.5	±15.9	±2.9	±2.1	±7.5	±16.0	±12.8	±27.7
Positive control	(μg/dish)								
NaN_3	1.5					1596.0 ±106.1			
2-AF	10.0		1351.3 ±105.8		3024.0 ±177.2		1842.7 ±167.2		
4-Nitro-0-phenylenediamine	20.0	1920.0 ±115.2		2529.3 ±184.4					
MMC	2.5							2751.3 ±94.2	
1,8-Dihydroxyanthraquinone	50.0								824.7 ±23.4

nb. Results are mean values of all 3 dishes

Table 3. Results of Eleotin Ames test: 2

		TA97		TA98		TA100		TA102	
Dose (mg/dish)		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
E	0.008	152.3	150.7	38.0	45.3	153.0	164.7	279.0	285.7
L		±12.6	±27.2	±2.0	±8.5	±12.1	±27.4	±13.1	±9.1
E	0.04	140.7	154.3	34.0	34.3	149.3	146.7	285.0	303.3
O		±10.3	±13.7	±1.5	±7.1	±4.5	±22.0	±19.0	±14.2
T	0.20	151.7	130.3	46.0	44.0	146.7	138.3	266.0	282.0
I		±13.8	±17.6	±13.2	±10.1	±22.0	±21.5	±15.9	±7.0
N	1.00	161.3	152.0	36.7	33.0	138.3	147.3	290.0	263.7
		±2.1	±20.8	±10.3	±7.9	±21.5	±8.7	±17.8	±21.8
	5.00	158.0	155.3	32.7	38.3	158.7	138.7	289.3	294.7
		±8.7	±14.2	±7.6	±3.5	±9.5	±8.1	±16.9	±13.3
Unprocessed-control		138.7	153.0	41.3	40.7	140.7	137.3	287.7	288.0
		±6.4	±20.8	±8.4	±8.3	±8.1	±12.3	±18.3	±10.0
Solvent-control		171.0	161.3	42.0	41.0	142.0	139.7	294.0	297.3
		±11.4	±3.8	±10.5	±1.0	±14.7	±7.2	±7.8	±12.1
Positive control	(μg/dish)								
NaN_3	1.5					1652.0 ±182.3			
2-AF	10.0		1213.3 ±110.1		2962.0 ±235.5		1962.7 ±150.4		
4-Nitro-0-phenylenediamine	20.0	1956.0 ±166.0		2389.3 ±150.2					
MMC	2.5							3036.7 ±72.5	
1,8-Dihydroxyanthraquinone	50.0								848.7± 115.0

nb. Results are mean values of all 3 dishes

2.2.2 Testing of occurrence of mutation of the micronucleus obtained from polychromatic erythrocytes in the bone marrow of mice: Table 4 shows that the % PCE for female baseline-control group and all Eleotin dose groups are between 49.8-52.4, while the % PCE for male baseline-control group and all Eleotin dose groups are between 50.0-53.1; no cell toxicity was observed under all Eleotin doses. The occurrences of mutation of the micronucleus of positive-control group (cyclophosphamide) for both male and female were higher than both the baseline-control group and Eleotin dose groups (Poisson Distribution, $p<0.01$). No statistical significance was observed with the occurrences of mutation f the micronucleus between Eleotin dose groups and baseline-control group ($p>0.05$). Results showed that Eleotin dose not cause mutation of the micronucleus of polychromatic erythrocytes.

Table 4. Effects of Eleotin on occurrences of mutation of the micronucleus obtained from polychromatic erythrocytes (PCE) in the bone marrow of mice

			PCE			Micronucleus		
Sex	Dose (g/kg.BW)	Number of subjects	Observed number of Erythrocytes	Number of PCE	%	Observed number of PCE	Number of micronucleus	% Occurrence ‰
Female	0.00[a]	5	1000	524	52.4	5000	12	2.4
	0.83	5	1000	498	49.8	5000	10	2.0
	2.50	5	1000	517	51.7	5000	8	1.6
	7.50	5	1000	509	50.9	5000	9	1.8
	40mg/kg.BW(CP)[b]	5	1000	446	44.6	5000	142	28.4
Male	0.00a	5	1000	531	53.1	5000	11	2.2
	0.83	5	1000	500	50.0	5000	12	2.4
	2.50	5	1000	513	51.3	5000	9	1.8
	7.50	5	1000	524	52.4	5000	10	2.0
	40mg/kg.BW(CP)[b]	5	1000	440	44.0	5000	156	31.2

[a]: Denotes comparison with each of the Eleotin processed-control groups, Poisson Distribution $p>0.05$

[b]: Denotes comparison with each of the Eleotin processed-control groups and baseline - control group, Poisson Distribution $p<0.01$

2.2.3 Testing for occurrences of sperm mutation in mice: Table 5 shows that the occurrences of sperm mutation was much higher in the positive-control group when compared with that of the baseline-control group and all of Eleotin dose groups (X^2-test: $p<0.01$); while no statistical significance was observed with the differences between all Eleotin dose groups and baseline-control group (X^2-test: $p>0.05$). Results showed that Eleotin does not cause sperm mutation in mice.

Table 5. Effects of Eleotin on occurrences of sperm mutation in mice

Dose (g/kg.BW)	Number of subjects	Number of sperms tested	Number of mutated sperms	% of sperm mutation
0.00[a]	5	5000	149	2.98
0.83	5	5000	118	2.36
2.50	5	5000	131	2.62
7.50	5	5000	103	2.06
40 mg/kg.BW(CP)[b]	5	5000	289	5.78

[a]: Denotes comparison with each of the Eleotin processed-control groups, X^2-test $p>0.05$

[b]: Denotes comparison with each of the Eleotin processed-control groups and baseline - control group, X^2-test $p<0.01$

2.3 Thirty-Day Eleotin intake test:

2.3.1 Growth and digestive efficiency & food usage: After 0.75, 1.50, and 3.00g/kg.BW of Eleotin were added to the normal diet of subjects for 30 days, activities and growth of all groups appeared normal, subjects were observed with healthy and vibrant hair. Tables 6, 7, and 8 show that there was no difference in the week-4 weight , total weight, total digestive efficiency & food usage of all Eleotin dose groups when compared with those of the control group ($p>0.05$).

Table 6. Effects of Eleotin on the weight of rats ($\overline{x}$ ± SD)

Sex	Dose (g/kg.BW)	Number of subjects	Initial weight (g)	Week 1 (g)	Week 2 (g)	Week 3 (g)	Week 4 (g)
Female	0.00	10	66.8±6.0	106.6±8.6	140.7±7.8	165.9±13.4	186.8±14.2
	0.75	10	66.0±4.6	107.7±8.6	145.4±12.4	173.4±15.3	194.4±19.4
	1.50	10	66.6±5.7	106.6±10.9	150.0±18.3	183.8±24.6	211.9±31.1
	3.00	10	65.8±5.4	106.5±9.6	136.6±14.0	159.2±15.7	182.1±18.0
Male	0.00	10	68.1±7.3	120.2±10.4	182.2±15.3	221.9±21.1	272.3±28.7
	0.75	10	68.3±4.5	114.6±7.7	171.9±15.6	214.9±18.7	254.6±25.2
	1.50	10	68.9±4.6	118.3±8.4	178.2±12.8	222.4±22.0	273.8±27.7
	3.00	10	68.1±4.3	112.9±9.8	164.3±14.9	203.2±20.8	247.9±25.0

Table 7. Effects of Eleotin on the weekly digestive efficiency & food usage of rats ($\overline{x}$ ± SD)

Sex	Dose (g/kg.BW)	Number of subjects	Weekly digestive efficiency and food usage (%)			
			Week 1	Week 2	Week 3	Week 4
Female	0.00	10	45.8±9.4	28.3±10.0	19.7±7.5	16.4±3.1
	0.75	10	48.3±11.7	28.6±7.1	20.8±4.3	15.4±6.6
	1.50	10	46.1±7.8	34.3±11.1	24.0±5.1	19.2±9.3
	3.00	10	49.2±9.6	25.8±11.9	16.4±4.0	16.0±4.3
Male	0.00	10	54.8±4.4	45.1±7.8	28.5±7.0	28.5±4.9
	0.75	10	49.2±4.4	40.6±8.4	28.9±6.5	24.0±11.0
	1.50	10	52.0±10.8	42.2±8.9	29.2±10.0	28.6±6.3
	3.00	10	56.1±13.3	40.2±7.7	26.1±6.9	28.8±10.8

Table 8. Effects of Eleotin on the food intake and total digestive efficiency & food usage of rats ($\overline{x}$ ± SD)

Sex	Dose (g/kg.BW)	Number of subjects	Total weight gain (g)	Total Food Intake (g)	Total digestive efficiency & food usage (%)
Female	0.00	10	120.0±17.2	464.2±19.8	25.9±4.1
	0.75	10	128.4±18.0	493.1±23.5	26.0±3.4
	1.50	10	145.3±29.8	502.3±22.4	28.9±5.4
	3.00	10	116.3±19.3	486.3±31.5	24.1±5.2
Male	0.00	10	204.2±25.6	549.8±22.8	37.1±4.4
	0.75	10	186.3±25.9	551.8±20.7	33.7±4.2
	1.50	10	204.9±29.9	571.3±27.0	36.0±5.8
	3.00	10	179.8±22.7	517.1±22.7	35.0±5.5

2.3.2 Haematology and biochemical indexes:

Tables 9 and 10 show that after 0.75, 1.50, and 3.00g/kg.BW of Eleotin were added to the normal diet of subjects for 30 days, other than the fact that the erythrocyte count of the female 1.50 and 3.00g/kg.BW Eleotin groups was higher than that of the control group ($p<0.05$, although differences did not reach biochemical significance when referenced with past experimental records within this laboratory), all remaining haematology indexes (leukocyte count, erythrocyte count, haemoglobin, etc)of all other Eleotin groups (both male and female subjects) are similar to those of the control group ($p>0.05$).

Table 9. Effects of Eleotin on haematology indices of rats ($\overline{x}$ ± SD)

Sex	Dose (g/kg.BW)	Number of subjects	Leukocyte count ($\times10^9$/L)	Erythrocyte count ($\times10^{12}$/L)	Haemoglobin (g/L)
Female	0.00	10	10.3±2.8	6.53±0.24	141.9±6.1
	0.75	10	10.6±1.8	6.50±0.23	143.0±5.7
	1.50	10	9.4±2.3	6.81±0.20*	146.7±4.3
	3.00	10	9.9±2.3	6.84±0.32*	144.3±5.9
Male	0.00	10	10.4±1.2	6.71±0.33	148.6±4.8
	0.75	10	9.6±2.3	6.86±0.11	148.3±2.4
	1.50	10	10.0±2.6	6.80±0.28	148.5±4.9
	3.00	10	10.8±3.3	6.73±0.34	143.8±6.0

*: Comparison with control group, $p<0.05$

Table 10. Effects of Eleotin on the different types of white blood cells of rats ($\overline{x}$ ± SD)

Sex	Dose (g/kg.BW)	Number of subjects	% Lymphocytes	% Neutriphils	% Other types
Female	0.00	10	80.4±4.8	12.5±5.2	7.1±1.0
	0.75	10	82.7±1.8	10.7±2.0	6.6±0.8
	1.50	10	83.7±3.2	10.4±2.4	5.9±1.2
	3.00	10	82.8±1.7	11.2±2.0	6.0±1.2
Male	0.00	10	83.4±3.2	10.6±2.5	6.0±1.3
	0.75	10	81.6±2.3	12.4±1.8	6.0±1.0
	1.50	10	81.7±2.0	11.8±2.4	6.5±0.8
	3.00	10	83.1±2.7	11.1±2.1	5.8±1.0

Table 11 shows that after 0.75, 1.50, and 3.00g/kg.BW of Eleotin were added to the normal diet of subjects for 30 days, other than the fact that the TP level of the male 0.75g/kg.BW Eleotin group was higher than that of the control group, and that the ALB level of the male 1.50 and 3.00g/kg.BW Eleotin groups was lower than that of the control group ($p<0.05$, although differences did not reach any significance when referenced with past experimental records within this laboratory), all remaining biochemical indexes, AST, ALT, urea (BUN), Cre, TC, TG, blood glucose level, TP, ALB of all other Eleotin groups (both male and female subjects) are similar to those of the control group ($p>0.05$).

Table 11. Biochemical indices of subjects (rats) after 30 days of Eleotin intake ($\overline{x}$ ± SD) [partial data]

Sex	Dose (g/kg.BW)	Number of subjects	Alanine aminotransferase (ALT) (U/L)	Aspartate aminotransferase (AST) (U/L)	Urea (BUN) (mmol/L)	Cre (umol/L)
Female	0.00	10	33.1±3.4	136.5±28.0	5.41±1.09	56.4±4.0
	0.75	10	34.6±4.4	139.5±22.4	5.22±0.71	55.1±3.3
	1.50	10	36.8±5.4	143.9±23.7	4.74±0.88	55.9±5.4
	3.00	10	35.5±2.5	145.1±13.7	4.79±0.98	54.5±2.4
Male	0.00	10	40.2±4.3	187.5±28.2	4.01±0.52	52.2±2.6
	0.75	10	40.7±10.6	203.6±51.9	4.28±0.56	52.8±3.0
	1.50	10	42.8±5.7	200.1±24.9	4.35±0.53	53.5±4.3
	3.00	10	42.1±4.5	185.0±26.9	3.99±0.82	52.5±4.9

Table 11 (continued). Biochemical indices of subjects (rats) after 30 days of Eleotin intake ($\overline{x}$ ± SD) [partial data]

Sex	Dose (g/kg.BW)	Number of subjects	TC (mmol/L)	TG (mmol/L)	Blood glucose level (mmol/L)	TP (g/L)	ALB (g/L)
Female	0.00	10	2.23±0.31	0.98±0.18	4.02±0.42	63.3±2.7	35.9±1.1
	0.75	10	2.14±0.41	0.97±0.22	4.23±0.46	65.1±4.2	36.4±1.2
	1.50	10	2.10±0.34	0.94±0.27	3.83±0.39	64.7±2.4	36.3±1.0
	3.00	10	2.35±0.40	1.21±0.53	3.87±0.59	63.4±2.4	36.0±0.9
Male	0.00	10	1.72±0.16	1.13±0.24	3.20±0.50	63.4±1.9	35.6±0.5
	0.75	10	2.06±0.27*	1.04±0.29	3.37±0.50	63.4±3.0	35.0±1.1
	1.50	10	1.94±0.28	1.41±0.46	3.45±0.45	65.0±1.2	35.4±0.5*
	3.00	10	1.97±0.26	1.18±0.42	3.56±0.52	62.6±2.1	35.3±0.7*

*: Comparison with control group, $p<0.05$

2.3.3 Anatomical pathology and histology:

Anatomical study of subjects found one case of testicular shrinkage in both testes of the same subjects from control group. No other abnormalities were observed. The differences in the ratio of mass of internal organs to body mass for all Eleotin dose groups when compared with that of the control group did not reach statistical significance (Table 12, $p>0.05$).

Histological studies on liver, kidneys, testes (or ovaries), stomach, duodenum, spleen: Liver: no structural changes occurred in hepatic lobules, liver cells were aligned, spot liver cell necrosis was observed in the control group (1/20 cases) and not observed in the 3.00g/kg.BW Eleotin group (0/20). No structural changes occurred in bile canaliculi and bile microcanaliculi. Kidneys: calcium and salt deposit observed in renal tubules of 1/20 control group subjects and 1/20 3.00g/kg.BW Eleotin group, renal tubule and renal cortex structures were clear, no changes, necrosis, or inflammation were observed with the epithelial cell of renal tubule. Testes: Testicular shrinkage observed in 1/20 control group subjects while no shrinkage (0/20) was observed with the 3.00g/kg.BW Eleotin group. All other groups produced healthy and some mature sperms. No structural changes were observed in the ovaries, ovarian follicles, and corpus luteum were identified. Stomach and duodenum mucosa remained the same, no signs of cell necrosis, ulceration, and inflammatory cell infiltration were observed. The structures of the red and white pulps of the spleen were clear; the number of lymphatic cells remains unchanged. The pathology observed in the liver, kidneys, and testes of the 3.00g/kg.BW Eleotin group does not reach statistical significance when compared with the control group; thus it was concluded to be due to natural histological changes, which occur in animals from time to time and unrelated to Eleotin. The results showed that Eleotin does

not cause pathological changes in liver, kidneys, testes (or ovaries), stomach, and duodenum.

Table 12. Effects of Eleotin on the mass of internal organs to body mass ratio of rats ($\overline{x}$ ± SD)

Sex	Dose (g/kg.BW)	Number of subjects	Final body mass (g)	Liver		Kidney	
				Mass (g)	Mass of organ:body mass (%)	Mass (g)	Mass of organ:body mass (%)
Female	0.00	10	171.3±13.0	5.58±0.41	3.26±0.20	1.53±0.17	0.90±0.07
	0.75	10	178.9±15.4	6.11±0.89	3.41±0.38	1.64±0.18	0.92±0.06
	1.50	10	184.8±15.2	6.06±0.68	3.27±0.16	1.66±0.15	0.90±0.08
	3.00	10	168.0±16.4	5.56±0.66	3.31±0.27	1.55±0.23	0.92±0.09
Male	0.00	10	256.8±27.6	7.90±1.19	3.07±0.23	2.22±0.33	0.86±0.05
	0.75	10	250.0±15.2	7.75±0.58	3.10±0.27	1.55±0.23	0.87±0.05
	1.50	10	252.0±26.6	7.69±0.93	3.05±0.24	2.18±0.27	0.87±0.13
	3.00	10	229.7±19.6	7.10±0.69	3.09±0.17	2.06±0.24	0.90±0.07

Table 12 (continued). Effects of Eleotin on the mass of internal organs to body mass ratio of rats ($\overline{x}$ ± SD)

Sex	Dose (g/kg.BW)	Number of subjects	Spleen		Testes	
			Mass (g)	Mass of organ:body mass (%)	Mass (g)	Mass of organ:body mass (%)
Female	0.00	10	0.48±0.11	0.28±0.07	-	-
	0.75	10	0.46±0.14	0.25±0.07	-	-
	1.50	10	0.47±0.09	0.26±0.05	-	-
	3.00	10	0.41±0.10	0.24±0.05	-	-
Male	0.00	10	0.68±0.14	0.26±0.05	2.71±0.42	1.06±0.15
	0.75	10	0.65±0.10	0.26±0.04	2.64±0.25	1.06±0.10
	1.50	10	0.65±0.15	0.26±0.05	2.65±0.22	1.06±0.13
	3.00	10	0.63±0.07	0.28±0.03	2.60±0.19	1.13±0.09

3 Conclusion:

3.1 Acute toxicity test (rats): subjects (male and female) were orally fed Eleotin and results determined a LD_{50} of greater than 10.0g/kg.BW. According to Acute Toxicity Classification, Eleotin was determined as non-toxic.

3.2 Genotoxicity assays: According to the results of the 3 types of genotoxicity assays performed (Ames test, testing for occurrences of mutation of the micronucleus obtained

from polychromatic erythrocytes in the bone marrow of mice, and testing for occurrences of sperm mutation in mice), Eleotin was not determined to be a source of genetic mutation.

3.3 Thirty-Day Eleotin intake test: After 0.75, 1.50, and 3.00g/kg.BW of Eleotin were added to the normal diet of subjects for 30 days, activities and growth of all groups appeared normal, subjects were observed with healthy and vibrant hair. There was no difference in weight, food intake, total digestive efficiency & food usage of all Eleotin dose groups (both male and female) when compared with those of the control group ($p>0.05$). Haematology and biochemical indexes: After 0.75, 1.50, and 3.00g/kg.BW of Eleotin were added to the normal diet of subjects for 30 days, other than the fact that the erythrocyte count of the female 1.50 and 3.00g/kg.BW Eleotin groups was higher than that of the control group ($p<0.05$, although differences did not reach biochemical significance when referenced with past experimental records within this laboratory), all remaining haematology indexes (leukocyte count, erythrocyte count, haemoglobin, etc) of all other Eleotin groups (both male and female subjects) are similar to those of the control group ($p>0.05$). Haematology: After 0.75, 1.50, and 3.00g/kg.BW of Eleotin were added to the normal diet of subjects for 30 days, other than the fact that the TP level of the male 0.75g/kg.BW Eleotin group was higher than that of the control group, and that the ALB level of the male 1.50 and 3.00g/kg.BW Eleotin groups was lower than that of the control group (($p<0.05$, although differences did not reach any significance when referenced with past experimental records within this laboratory), all remaining biochemical indexes, AST, ALT, urea (BUN), Cre, TC, TG, blood glucose level, TP, ALB of all other Eleotin groups (both male and female subjects) are similar to those of the control group ($p>0.05$). The differences in the ratio of mass of internal organs to body mass for all Eleotin dose groups when compared with that of the control group did not reach statistical significance ($p>0.05$). Results of the pathological test showed that Eleotin does not cause pathological changes.