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:	October 22, 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 + 2000g
Sample Received on:	May 11th, 2004
Testing Criteria:	Sample's functions as a supplement in decreasing blood glucose level (animal-testing)
Testing Reference:	Natural Health Products Testing and Evaluation Guidelines- 2003

Testing Results:

The results showed that Eleotin had no effect on the blood glucose levels of the normal subjects (laboratory mice) after fasting ($p>0.05$). Compared with the glucose-matched control group, Eleotin effectively lowered the blood glucose levels ($p<0.05$ or $p<0.01$) of empty-stomach mice with previously high blood glucose levels induced by Alloxan in all Eleotin dose groups (high dose, medium dose, and low dose). After oral feeding of glucose to all Eleotin dose groups, the blood glucose levels of these groups were still lower than that of the glucose-matched control group ($p<0.01$). According to the standards set by Natural Health Products Testing and Evaluation Guidelines- 2003, the testing of whether Eleotin is effective in supplementing the decrease of blood glucose level in an animal experiment returned positive results. (end of page)

Eleotin's functions as a supplement in decreasing blood glucose level: Animal-Testing 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, an equivalent of 0.03g/kg.BW (Calculations based on an adult body weight of 60kg).

1.2 Subjects: 6-week old male mice from Kunming, each weighing approximately 28g, B-grade, purchased from the Chinese People's Liberation Army's Military Medical Science Laboratory, Animal Testing Centre. License# SCXK-(Military) 2002-001.

1.3 Instrument and Testing Reagent: Glucotrend blood glucose monitor, purchased from Roche; Alloxan, purchased from Sigma

1.4 Experimental Procedure:

1.4.1 The effects of Eleotin on the blood glucose levels of normal mice: 24 healthy adult male mice were selected, after five hours of fasting (with access to water) the blood glucose levels of empty-stomach mice were measured via blood samples taken from the tail. According to their blood glucose levels, subjects were then randomly assigned into two groups: natural glucose-matched control group (blood glucose level at baseline) and high dose Eleotin group, each with 12 subjects. The natural glucose-matched control group was intragastrically given distilled water daily for 30 days, while the high dose Eleotin group was given 0.9g/kg.BW of Eleotin, equivalent to 30 times that of the recommended dose for human adults (0.03g/kg.BW). At the end of this period, all subjects were again deprived of food for five hours (with access to water), following which the blood glucose levels were measured to observe the effects of Eleotin on the blood glucose levels of normal subjects on an empty stomach.

1.4.2 Effects of Eleotin on the blood glucose levels and glucose tolerance of empty-stomach mice with previously high blood glucose levels induced by Alloxan: After fasting for 24 hours, male mice were intravenously injected with 45mg/kg.BW of soluble Alloxan in the tail at 0.2ml/20g.BW. Five days later and after five hours of fasting, blood glucose levels of empty-stomach subjects were measured via blood samples taken from the tail. Those with levels higher than 10 mmol/L were selected, and according to blood glucose levels, divided into glucose-matched control group and low, medium, and high dose Eleotin groups. The Eleotin groups (experimental groups) were intragastrically given Eleotin at 5, 10,and 30 times the human recommended dose, at 0.15, 0.30, and 0.90/ kg.BW respectively. After daily intragastric feeding of Eleotin for 30 days, the subjects were tested for glucose-tolerance: after all subjects were food-deprived for five hours, the experimental groups were given different concentrations of Eleotin, while the glucose-matched control group was given the equivalent volume of water. After 20 minutes, all groups were orally fed 2.5g/kg.BW of 25 % liquid glucose at 0.1ml/10g.BW. Blood glucose levels were measured via blood

samples taken from the tail after 0, 0.5, and 2 hours. The changes in blood glucose level of the glucose-matched control group were compared with that of the experimental groups at different time intervals.

1.5 Statistical Analysis: Variance analysis of the data obtained was performed using SPSS software. When homogeneity of variance is achieved ($p>0.05$), no statistical significance is reached; however, when the mean values of the glucose-matched control group were compared with those of the experimental groups, no homogeneity of variance was observed ($p\leq0.05$), with differences reaching statistical significance.

1.6 Results assessment: Of the two criteria tested (empty-stomach blood glucose level and glucose tolerance), one returned positive results. No effects were observed with respect to the empty-stomach blood glucose level of normal subjects. Thus it can be concluded that the testing of Eleotin's functions as a supplement in decreasing blood glucose level in animals returned positive results.

2 Results:

2.1 Effects of Eleotin on the body weight of normal subjects:

According to Table 1, there is no statistical significance ($p>0.05$) between the high dose Eleotin group and the natural glucose-matched control group.

Table 1. Body weight (g) of normal mice at start, mid-period, and end of testing

Groups	Number of Subjects	Body weight (g) Start	Mid-period	End
Natural glucose-matched	12	33.8 ± 1.7	40.4 ± 1.9	45.6 ± 2.9
High dose (Eleotin)	12	33.4 ± 2.6	41.0 ± 3.6	46.8 ± 3.3

2.2 Effects of Eleotin on the blood glucose level of normal empty-stomach subjects:

Table 2 shows that there is no statistical significance ($p>0.05$) when comparing the blood glucose levels of natural glucose-matched control group with that of the high dose group both before and after testing.

Table 2. Effects of Eleotin on the blood glucose level of normal empty-stomach subjects

Groups	Dose (g/kg.BW)	Empty-stomach blood glucose value (mmol/L)			
		Before testing	P-value	After testing	P-value
Natural glucose-matched	0.00	7.1 ± 0.8	–	7.3 ± 0.8	–
High dose (Eleotin)	0.90	7.2 ± 1.5	0.880	7.2 ± 1.2	0.743

2.3 Effects of Eleotin on the body weight of subjects with high blood glucose level induced by Alloxan:

Table 3 shows that there is no statistical significance ($p>0.05$) when comparing the body weight of glucose-matched control group to that of the other three Eleotin dose groups.

Table 3. Effects of Eleotin on the body weight of subjects with high blood glucose level

Groups	Number of subjects	Body weight (g)		
		Start	Mid-period	End
Glucose-matched control	12	32.5 ± 2.8	39.7 ± 3.6	44.5 ± 3.9
(Eleotin) Low dose	12	32.7 ± 2.5	40.3 ± 2.8	46.1 ± 3.1
Medium dose	12	32.3 ± 2.2	40.2 ± 2.7	45.9 ± 4.1
High dose	12	33.6 ± 2.2	41.0 ± 4.7	45.7 ± 5.5

2.4 Effects of Eleotin on the blood glucose levels of empty-stomach subjects with previously high blood glucose levels induced by Alloxan:

Table 4 shows no statistical significance between the blood glucose levels of subjects in all groups prior to testing ($p>0.05$). After testing, we found that Eleotin dose at all levels evidently decreased the blood glucose levels of empty-stomach subjects with previously high blood glucose levels induced by Alloxan when the results were compared with the glucose-matched control group ($p<0.05$ or $p<0.01$). The differences in blood glucose levels of all three Eleotin dose groups when compared with the glucose-matched control group reached statistical significance ($p<0.01$).

Table 4. Effects of Eleotin on the blood glucose levels of empty-stomach subjects

Groups	Dose (g/kg.BW)	# of subjects	Empty-stomach blood glucose value (mmol/L)		
			Before testing	After testing	Difference
Glucose-matched control	0.00	12	16.4 ± 2.5	18.9 ± 2.4	2.4 ± 1.9
(Eleotin) Low dose	0.15	12	16.3 ± 2.4	16.4 ± 2.7*	0.2 ± 1.9**
Medium dose	0.30	12	16.8 ± 2.3	16.7 ± 2.5*	-0.2 ± 1.9**
High dose	0.90	12	16.5 ± 2.0	15.2 ± 2.4**	-1.3 ± 1.7**

Compared with glucose-matched control group: $^{**}p<0.01$

2.5 Effects of Eleotin on the glucose tolerance level of subjects with previously high blood glucose level induced by Alloxan:

Table 5 and 6 show that after subjects with previously high blood glucose levels induced by Alloxan were orally fed liquid glucose (2.5g/kg.BW), Eleotin evidently decreased the blood glucose level of the Eleotin groups (low, medium, high dose) after 0.5 and 2 hours, as seen when compared with values obtained from the glucose-matched control group; the differences also reached statistical significance after performing statistical analysis ($p<0.05$ or $p<0.01$). After all groups were fed liquid glucose, the area under the curve for the Eleotin groups (low, medium, high dose) was also smaller than that of the glucose-matched control group, with differences reaching statistical significance ($p<0.01$).

Table 5. Effects of Eleotin on the blood glucose level of subjects with previously high blood glucose level (induced by Alloxan) and after oral administration of liquid glucose

Groups	Number of subjects	Blood glucose level (mmol/L)		
		0 h	0.5 h	2 h
Glucose-matched control	12	18.9 ± 2.4	25.6 ± 3.1	20.2 ± 2.8
(Eleotin) Low dose	12	16.4 ± 2.7*	21.3 ± 3.3**	17.2 ± 2.6**
Medium dose	12	16.7 ± 2.5*	21.6 ± 2.9**	17.5 ± 2.2*
High dose	12	15.2 ± 2.4**	20.5 ± 2.9**	15.5 ± 2.8**

Compared with glucose-matched control group: * $p<0.05$, ** $p<0.01$

Table 6. Effects of Eleotin on the area under the blood glucose curve for subjects with previously high blood glucose levels induced by Alloxan

Groups	Dose (g/kg.BW)	# of subjects	Area under the curve
Glucose-matched control	0.00	12	45.5 ± 5.7
Low dose	0.15	12	38.3 ± 5.6**
Medium dose	0.30	12	38.9 ± 5.1**
High dose	0.90	12	35.9 ± 5.4**

Compared with glucose-matched control group: ** $p<0.01$

3 Conclusion: The results showed that Eleotin had no effect on the blood glucose level of the normal subjects (laboratory mice) after fasting ($p>0.05$). No statistical significance ($p>0.05$) was observed in the blood glucose levels of all groups of subjects with high blood glucose levels prior to testing. After testing, compared with the glucose-matched control group, Eleotin (low, medium, high dose) evidently decreased the blood glucose levels of empty-stomach subjects with previously high blood glucose levels induced by

Alloxan; statistical significance is observed ($p<0.01$) when comparing the differences in blood glucose levels prior to testing and after testing of low, medium, and high Eleotin dose groups with that of the glucose-matched control group. After subjects with previously high blood glucose level induced by Alloxan were orally fed liquid glucose (2.5g/kg.BW), Eleotin evidently decreased the blood glucose level of the Eleotin groups (low, medium, high dose) after 0.5 and 2 hours, as seen when compared with values obtained from the glucose-matched control group, the differences also reached statistical significance after performing statistical analysis ($p<0.05$ or $p<0.01$). After all groups were fed liquid glucose, the area under the curve for the Eleotin groups (low, medium, high dose) was also smaller than that of the glucose-matched control group, reaching statistical significance ($p<0.01$). According to the standards set by Natural Health Products Testing and Evaluation Guidelines - 2003, the testing of whether Eleotin is effective in supplementing the decrease of blood glucose level in an animal experiment returned positive results. (End of page)