

# LECTRANAL® - PRECLINICAL TOXICITY STUDY

## SUMMARY

To determine the safety profile of allergy-relieving ingredient Lectranal®, a multiple-dose, 28-day toxicity study was performed on mice. The study was performed at the Ruđer Bošković Institute in Zagreb according to OECD protocol 407. Since Lectranal® requires an enteric delivery, in this experiment it was administered either by gastric or enteric gavage at three different dosage levels.

No mortality, adverse reactions, or behavioral changes were observed in any of the groups. There was no significant weight change in any of the animals. Feed and water consumption were unchanged compared to the controls. Urinalysis, haematology, and biochemistry results revealed no significant difference between the treated and control groups. No changes were revealed by necropsy and there was no difference in organ weight between the treated and control animals. Histology results were normal and similar to the controls. There were no toxicity effects of any kind as result of Lectranal® application.

Taken together, these results show that Lectranal® has a good safety profile.

## OBJECTIVE

The focus of this study was to determine the safety and toxicity aspects of Lectranal® following a 28-day oral administration.

## MATERIALS AND METHODS

### Test animals and conditions

Because of the previous studies, mice were chosen as test animals. Ninety-six CBA/HZgr (H-2<sup>k</sup>) mice of both sexes were obtained from the Ruđer Bošković Institute. After a 14-day acclimatisation period, the animals were selected at random and divided into 14 groups, each group consisting of 6 animals of the same sex, except control groups which consisted of 12 animals. The initial weight of the male mice was 26 to 33 g, and female mice weighed between 22 and 28 g. The animals were kept 3 per cage (Type PC3108, EHRET cage) and given access to the laboratory feed Mucedola (Settino Milanese) and water *ad libitum*.

### Dosage

Three different levels of Lectranal® were administered by either gastric or enteric gavage for 28 consecutive days, and 24 animals were used as a control group.

group	Number of animals	Dosage (mg/mice)	Mode of administration
FEMALE ANIMALS			
Group F-5 <sub>E</sub>	6	5	enteric gavage
Group F-50 <sub>E</sub>	6	50	enteric gavage
Group F-75 <sub>E</sub>	6	75	enteric gavage
Group F-5 <sub>G</sub>	6	5	gastric gavage
Group F-50 <sub>G</sub>	6	50	gastric gavage
Group F-75 <sub>G</sub>	6	75	gastric gavage
Group F-Control	12	0	-
MALE ANIMALS			
Group M-5 <sub>E</sub>	6	5	enteric gavage
Group M-50 <sub>E</sub>	6	50	enteric gavage
Group M-75 <sub>E</sub>	6	75	enteric gavage
Group M-5 <sub>G</sub>	6	5	gastric gavage
Group M-50 <sub>G</sub>	6	50	gastric gavage
Group M-75 <sub>G</sub>	6	75	gastric gavage
Group M-Control	12	0	-

## Research protocol

Mortality/morbidity and general clinical status were evaluated each day. A detailed clinical examination as well as urine collection and analysis was performed once a week. Body weight, feed, and water intake was measured weekly. In the fourth week of exposure, reactivity to stimuli and motor activity was assessed.

On the 29<sup>th</sup> day the animals were sacrificed in a CO<sub>2</sub> atmosphere, blood samples were collected, and clinical biochemistry and haematology tests were performed. All animals were subjected to detailed gross necropsy, and selected organs were trimmed and weighed.

Furthermore, in the control and high dose groups (F-75<sub>E</sub>, F-75<sub>G</sub>, M-75<sub>E</sub>, M-75<sub>G</sub>), 4- to 6- $\mu$ m sections of the preserved target organs were collected, processed by standard histologic methods, and embedded in paraffin. Slides were prepared and stained with hemalaun-eosine.

## Statistics

All data were processed with an SPSS program and analyzed using ANOVA. All values are reported as mean $\pm$ S.D. Statistical significance was set at  $p < 0.05$ .

## RESULTS

### Effects of Lectranal<sup>®</sup> on mortality, general clinical status, and behavior

There was no mortality nor any unusual observations in any of the examined groups. A detailed clinical examination performed once a week showed no changes in skin, fur, eyes, mucous membranes, secretion and excretion, or autonomic activity (lacrimation, piloerection, pupil size, breathing). There were no changes in posture, movements, reaction to stimuli, or motor activity. No unusual behavior was noticed.

### Effects of Lectranal<sup>®</sup> on Body Weight and Feed and Water Intake

The changes in body weight in the Lectranal<sup>®</sup> and control groups are presented in **Table 1**. A gain in body weight was noticed in both the Lectranal<sup>®</sup> and control groups. After 28 days of Lectranal<sup>®</sup> supplementation in both female and male animals, there was no significant difference in body weight compared to initial body weight or compared to control group.

WEEKLY BODY WEIGHT						
week	female			male		
	group	Mean(g) $\pm$ SD		group	Mean(g) $\pm$ SD	
0	F-5 <sub>E</sub>	26.33 $\pm$	1.751	M-5 <sub>E</sub>	28.67 $\pm$	0.516
	F-50 <sub>E</sub>	25.33 $\pm$	0.816	M-50 <sub>E</sub>	31.50 $\pm$	0.837
	F-75 <sub>E</sub>	24.83 $\pm$	0.753	M-75 <sub>E</sub>	30.50 $\pm$	2.074
	F-5 <sub>G</sub>	25.67 $\pm$	2.160	M-5 <sub>G</sub>	29.33 $\pm$	1.751
	F-50 <sub>G</sub>	25.67 $\pm$	1.966	M-50 <sub>G</sub>	28.17 $\pm$	0.753
	F-75 <sub>G</sub>	24.50 $\pm$	0.837	M-75 <sub>G</sub>	29.33 $\pm$	1.033
	F-Control	25.50 $\pm$	2.111	M-Control	29.50 $\pm$	2.195
1	F-5 <sub>E</sub>	22.83 $\pm$	1.602	M-5 <sub>E</sub>	27.00 $\pm$	0.632
	F-50 <sub>E</sub>	22.83 $\pm$	1.835	M-50 <sub>E</sub>	30.00 $\pm$	0.632
	F-75 <sub>E</sub>	22.33 $\pm$	0.516	M-75 <sub>E</sub>	29.83 $\pm$	1.602
	F-5 <sub>G</sub>	22.00 $\pm$	1.095	M-5 <sub>G</sub>	27.67 $\pm$	2.066
	F-50 <sub>G</sub>	22.17 $\pm$	0.983	M-50 <sub>G</sub>	27.50 $\pm$	1.378
	F-75 <sub>G</sub>	22.17 $\pm$	1.169	M-75 <sub>G</sub>	28.50 $\pm$	1.225
	F-Control	23.17 $\pm$	1.267	M-Control	28.75 $\pm$	2.094
2	F-5 <sub>E</sub>	22.83 $\pm$	2.483	M-5 <sub>E</sub>	26.83 $\pm$	1.169
	F-50 <sub>E</sub>	22.50 $\pm$	1.378	M-50 <sub>E</sub>	30.33 $\pm$	0.816
	F-75 <sub>E</sub>	23.17 $\pm$	1.169	M-75 <sub>E</sub>	29.50 $\pm$	1.517
	F-5 <sub>G</sub>	22.67 $\pm$	0.516	M-5 <sub>G</sub>	28.17 $\pm$	1.602
	F-50 <sub>G</sub>	22,67 $\pm$	1.033	M-50 <sub>G</sub>	28.00 $\pm$	1.789
	F-75 <sub>G</sub>	21.67 $\pm$	1.966	M-75 <sub>G</sub>	28.17 $\pm$	1.472
	F-Control	23.17 $\pm$	1.697	M-Control	29.33 $\pm$	1.969

3	F-5 <sub>E</sub>	23.00±	1.789	M-5 <sub>E</sub>	27.50±	1.378
	F-50 <sub>E</sub>	23.33±	1.033	M-50 <sub>E</sub>	30.33±	1.366
	F-75 <sub>E</sub>	23.17±	1.329	M-75 <sub>E</sub>	30.33±	1.633
	F-5 <sub>G</sub>	23.67±	0.516	M-5 <sub>G</sub>	28.50±	1.975
	F-50 <sub>G</sub>	23.33±	0.816	M-50 <sub>G</sub>	28.00±	1.897
	F-75 <sub>G</sub>	22.50±	0.837	M-75 <sub>G</sub>	29.33±	1.366
	F-Control	23.92±	1.084	M-Control	29.83±	1.946
4	F-5 <sub>E</sub>	23.00±	1.549	M-5 <sub>E</sub>	27.33±	0.816
	F-50 <sub>E</sub>	23.83±	1.329	M-50 <sub>E</sub>	30.50±	1.378
	F-75 <sub>E</sub>	23.33±	1.211	M-75 <sub>E</sub>	30.50±	2.258
	F-5 <sub>G</sub>	24.33±	1.033	M-5 <sub>G</sub>	29.00±	2.366
	F-50 <sub>G</sub>	23.17±	0.983	M-50 <sub>G</sub>	27.50±	3.728
	F-75 <sub>G</sub>	23.00±	0.894	M-75 <sub>G</sub>	28.50±	1.643
	F-Control	24.08±	1.165	M-Control	29.33±	1.923

**Table 1.** Dose and Time-Dependent Effects of Lectranal® on Body Weight

Amount of feed intake in the Lectranal® and control groups is presented in **Table 2**. During 28 days of the experiment, the amounts of consumed feed in the Lectranal® and control groups were similar. There was no excessive intake or refusal of feed.

FEED INTAKE						
	female			male		
week	group	Mean(g)±SD		group	Mean(g)±SD	
1	F-5 <sub>E</sub>	2.70±	0.00	M-5 <sub>E</sub>	2.20±	0.00
	F-50 <sub>E</sub>	2.80±	0.00	M-50 <sub>E</sub>	3.10±	0.00
	F-75 <sub>E</sub>	2.60±	0.00	M-75 <sub>E</sub>	2.90±	0.00
	F-5 <sub>G</sub>	2.80±	0.00	M-5 <sub>G</sub>	3.10±	0.00
	F-50 <sub>G</sub>	3.10±	0.00	M-50 <sub>G</sub>	3.00±	0.00
	F-75 <sub>G</sub>	3.00±	0.00	M-75 <sub>G</sub>	3.30±	0.00
	F-Control	2.05±	0.0522	M-Control	2.25±	0.0522
2	F-5 <sub>E</sub>	2.70±	0.00	M-5 <sub>E</sub>	1.90±	0.00
	F-50 <sub>E</sub>	2.90±	0.00	M-50 <sub>E</sub>	3.20±	0.00
	F-75 <sub>E</sub>	2.30±	0.00	M-75 <sub>E</sub>	2.90±	0.00
	F-5 <sub>G</sub>	2.90±	0.00	M-5 <sub>G</sub>	2.80±	0.00
	F-50 <sub>G</sub>	2.60±	0.00	M-50 <sub>G</sub>	2.90±	0.00
	F-75 <sub>G</sub>	2.40±	0.00	M-75 <sub>G</sub>	3.25±	0.00
	F-Control	2.85±	0.1567	M-Control	2.85±	0.1567
3	F-5 <sub>E</sub>	1.80±	0.00	M-5 <sub>E</sub>	2.70±	0.00
	F-50 <sub>E</sub>	2.90±	0.00	M-50 <sub>E</sub>	2.40±	0.00
	F-75 <sub>E</sub>	2.70±	0.00	M-75 <sub>E</sub>	3.10±	0.00
	F-5 <sub>G</sub>	3.10±	0.00	M-5 <sub>G</sub>	2.90±	0.00
	F-50 <sub>G</sub>	2.90±	0.00	M-50 <sub>G</sub>	2.90±	0.00
	F-75 <sub>G</sub>	3.00±	0.00	M-75 <sub>G</sub>	3.20±	0.00
	F-Control	2.75±	0.3656	M-Control	3.00±	0.1044
4	F-5 <sub>E</sub>	2.20±	0.00	M-5 <sub>E</sub>	2.50±	0.00
	F-50 <sub>E</sub>	2.50±	0.00	M-50 <sub>E</sub>	3.20±	0.00
	F-75 <sub>E</sub>	2.80±	0.00	M-75 <sub>E</sub>	2.50±	0.00
	F-5 <sub>G</sub>	2.80±	0.00	M-5 <sub>G</sub>	3.40±	0.00
	F-50 <sub>G</sub>	2.20±	0.00	M-50 <sub>G</sub>	2.60±	0.00
	F-75 <sub>G</sub>	2.60±	0.00	M-75 <sub>G</sub>	2.70±	0.00
	F-Control	2.55±	0.2611	M-Control	2.50±	0.1044

**Table 2.** Dose and Time-Dependent Effects of Lectranal® on feed intake

The water intake of the Lectranal® and control groups is presented in **Table 3**. There were no significant differences in water intake between the Lectranal® and control groups.

WATER INTAKE						
week	female			male		
	group	Mean(ml)±SD		group	Mean(ml)±SD	
1	F-5 <sub>E</sub>	1.30±	0.00	M-5 <sub>E</sub>	3.00±	0.00
	F-50 <sub>E</sub>	1.50±	0.00	M-50 <sub>E</sub>	3.20±	0.00
	F-75 <sub>E</sub>	1.70±	0.00	M-75 <sub>E</sub>	2.50±	0.00
	F-5 <sub>G</sub>	1.00±	0.00	M-5 <sub>G</sub>	1.70±	0.00
	F-50 <sub>G</sub>	1.30±	0.00	M-50 <sub>G</sub>	2.30±	0.00
	F-75 <sub>G</sub>	2.30±	0.00	M-75 <sub>G</sub>	3.70±	0.00
	F-Control	1.70±	0.00	M-Control	1.45±	0.2611
2	F-5 <sub>E</sub>	1.50±	0.00	M-5 <sub>E</sub>	2.30±	0.00
	F-50 <sub>E</sub>	1.70±	0.00	M-50 <sub>E</sub>	1.70±	0.00
	F-75 <sub>E</sub>	1.70±	0.00	M-75 <sub>E</sub>	1.70±	0.00
	F-5 <sub>G</sub>	1.80±	0.00	M-5 <sub>G</sub>	2.10±	0.00
	F-50 <sub>G</sub>	1.80±	0.00	M-50 <sub>G</sub>	1.80±	0.00
	F-75 <sub>G</sub>	2.30±	0.00	M-75 <sub>G</sub>	2.30±	0.00
	F-Control	2.75±	0.2611	M-Control	2.15±	0.1567
3	F-5 <sub>E</sub>	1.20±	0.00	M-5 <sub>E</sub>	2.50±	0.00
	F-50 <sub>E</sub>	3.00±	0.00	M-50 <sub>E</sub>	2.20±	0.00
	F-75 <sub>E</sub>	2.60±	0.00	M-75 <sub>E</sub>	2.90±	0.00
	F-5 <sub>G</sub>	3.20±	0.00	M-5 <sub>G</sub>	2.90±	0.00
	F-50 <sub>G</sub>	3.10±	0.00	M-50 <sub>G</sub>	2.80±	0.00
	F-75 <sub>G</sub>	3.30±	0.00	M-75 <sub>G</sub>	2.50±	0.00
	F-Control	2.85±	0.1567	M-Control	2.85±	0.1567
4	F-5 <sub>E</sub>	1.70±	0.00	M-5 <sub>E</sub>	2.50±	0.00
	F-50 <sub>E</sub>	3.00±	0.00	M-50 <sub>E</sub>	2.90±	0.00
	F-75 <sub>E</sub>	3.30±	0.00	M-75 <sub>E</sub>	2.50±	0.00
	F-5 <sub>G</sub>	2.90±	0.00	M-5 <sub>G</sub>	3.30±	0.00
	F-50 <sub>G</sub>	3.20±	0.00	M-50 <sub>G</sub>	3.00±	0.00
	F-75 <sub>G</sub>	2.50±	0.00	M-75 <sub>G</sub>	2.50±	0.00
	F-Control	3.065±	0.2454	M-Control	2.75±	0.0522

**Table 3.** Dose and Time-Dependent Effects of Lectranal® on water intake

### Effects of Lectranal® on urine excretion and urinalysis

Urine was collected and analysed once a week, after a 24-hour stay in metabolic cages. The amount of excreted urine in the treated and control groups is presented in **Table 4**. There was no significant difference in the amount of excreted urine between the control and Lectranal® groups. The PH of the urin was between 6 and 7 in both the control and treated groups. There was no difference in the specific gravity of the urine between the control and treated groups. The presence of proteins, acetone, glucose, and blood was tested in the urine using a Combur 10 Test M (Roche). During the entire experiment none of the above parameters were detected.

AMOUNT OF EXCRETED URINE						
week	female			male		
	group	Mean(ml)±SD		group	Mean(ml)±SD	
1	F-5 <sub>E</sub>	1.30±	0.00	M-5 <sub>E</sub>	1.50±	0.00
	F-50 <sub>E</sub>	1.00±	0.00	M-50 <sub>E</sub>	1.70±	0.00
	F-75 <sub>E</sub>	1.00±	0.00	M-75 <sub>E</sub>	1.70±	0.00
	F-5 <sub>G</sub>	1.00±	0.00	M-5 <sub>G</sub>	1.70±	0.00
	F-50 <sub>G</sub>	1.30±	0.00	M-50 <sub>G</sub>	1.10±	0.00
	F-75 <sub>G</sub>	1.30±	0.00	M-75 <sub>G</sub>	1.30±	0.00
	F-Control	1.15±	0.1567	M-Control	1.55±	0.2611

2	F-5 <sub>E</sub>	1.50±	0.00	M-5 <sub>E</sub>	1.80±	0.00
	F-50 <sub>E</sub>	1.50±	0.00	M-50 <sub>E</sub>	1.70±	0.00
	F-75 <sub>E</sub>	1.70±	0.00	M-75 <sub>E</sub>	1.70±	0.00
	F-5 <sub>G</sub>	1.70±	0.00	M-5 <sub>G</sub>	1.80±	0.00
	F-50 <sub>G</sub>	1.60±	0.00	M-50 <sub>G</sub>	1.50±	0.00
	F-75 <sub>G</sub>	1.30±	0.00	M-75 <sub>G</sub>	2.00±	0.00
	F-Control	1.10±	0.1044	M-Control	1.65±	0.1567
3	F-5 <sub>E</sub>	1.20±	0.00	M-5 <sub>E</sub>	1.30±	0.00
	F-50 <sub>E</sub>	1.20±	0.00	M-50 <sub>E</sub>	1.30±	0.00
	F-75 <sub>E</sub>	1.30±	0.00	M-75 <sub>E</sub>	1.70±	0.00
	F-5 <sub>G</sub>	1.50±	0.00	M-5 <sub>G</sub>	1.50±	0.00
	F-50 <sub>G</sub>	1.70±	0.00	M-50 <sub>G</sub>	1.50±	0.00
	F-75 <sub>G</sub>	1.20±	0.00	M-75 <sub>G</sub>	1.20±	0.00
	F-Control	1.10±	0.1044	M-Control	1.40±	0.1044
4	F-5 <sub>E</sub>	1.00±	0.00	M-5 <sub>E</sub>	1.20±	0.00
	F-50 <sub>E</sub>	1.00±	0.00	M-50 <sub>E</sub>	1.50±	0.00
	F-75 <sub>E</sub>	1.70±	0.00	M-75 <sub>E</sub>	1.50±	0.00
	F-5 <sub>G</sub>	1.30±	0.00	M-5 <sub>G</sub>	1.80±	0.00
	F-50 <sub>G</sub>	1.30±	0.00	M-50 <sub>G</sub>	1.30±	0.00
	F-75 <sub>G</sub>	1.20±	0.00	M-75 <sub>G</sub>	1.30±	0.00
	F-Control	1.18±	0.0209	M-Control	1.15±	0.1567

**Table 4.** Effects of Lectranal® on urine excretion during 24-hour stay in metabolic cages

#### Effects of Lectranal® on haematological and biochemical parameters

Haematological parameters were determined from the blood samples using the Advia 60 blood cell counter (Siemens Medical Diagnostics). **Table 5** presents results white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), hematocrit (HCT), lymphocyte count (Lym), and monocyte count (Mon) in the control and treated groups.

Biochemical parameters were determined from serum samples using the Olympus Au600 (Olympus Optical Co.). **Table 6** and **Table 7** present results of the total serum protein, total albumin, glucose, cholesterol, urea, creatinine, aspartate aminotransferase, calcium, and potassium tests. No significant differences were observed in any of the treatment groups as compared to the control group.

HAEMATOLOGICAL PARAMETERS						
parameter	female			male		
	group	Mean±SD		group	Mean±SD	
HCT %	F-5 <sub>E</sub>	26.333±	3.8443	M-5 <sub>E</sub>	25.100±	4.4394
	F-50 <sub>E</sub>	22.750±	7.9500	M-50 <sub>E</sub>	26.683±	1.5263
	F-75 <sub>E</sub>	30.350±	1.8426	M-75 <sub>E</sub>	27.583±	3.0779
	F-5 <sub>G</sub>	24.883±	2.5436	M-5 <sub>G</sub>	26.500±	3.0906
	F-50 <sub>G</sub>	22.483±	4.3130	M-50 <sub>G</sub>	26.617±	1.8883
	F-75 <sub>G</sub>	26.183±	5.1316	M-75 <sub>G</sub>	28.617±	3.3090
	F-Control	24.383±	5.2091	M-Control	26.042±	4.1485
Hb g/L	F-5 <sub>E</sub>	91.33±	5.428	M-5 <sub>E</sub>	90.17±	7.360
	F-50 <sub>E</sub>	89.67±	11.860	M-50 <sub>E</sub>	101.83±	4.997
	F-75 <sub>E</sub>	101.83±	5.154	M-75 <sub>E</sub>	103.67±	6.439
	F-5 <sub>G</sub>	92.83±	3.430	M-5 <sub>G</sub>	97.33±	6.314
	F-50 <sub>G</sub>	91.83±	7.679	M-50 <sub>G</sub>	101.33±	4.131
	F-75 <sub>G</sub>	103.17±	9.368	M-75 <sub>G</sub>	99.33±	5.046
	F-Control	92.25±	9.762	M-Control	95.50±	7.693
RBC ×10 <sup>12</sup> /L	F-5 <sub>E</sub>	5.0850±	0.21998	M-5 <sub>E</sub>	5.0100±	0.55390
	F-50 <sub>E</sub>	5.0133±	1.06611	M-50 <sub>E</sub>	5.3333±	0.45933
	F-75 <sub>E</sub>	6.2850±	0.59031	M-75 <sub>E</sub>	5.9317±	0.50301
	F-5 <sub>G</sub>	4.9217±	0.54839	M-5 <sub>G</sub>	5.2617±	0.97130
	F-50 <sub>G</sub>	4.9283±	0.77381	M-50 <sub>G</sub>	5.5950±	0.29575
	F-75 <sub>G</sub>	5.8267±	0.79984	M-75 <sub>G</sub>	5.3717±	0.44038
	F-Control	5.2342±	0.94992	M-Control	5.5592±	0.51697

<b>WBC</b> ×10 <sup>9</sup> /L	F-5 <sub>E</sub>	3.933±	0.4502	M-5 <sub>E</sub>	4.550±	0.9094
	F-50 <sub>E</sub>	4.983±	0.6646	M-50 <sub>E</sub>	4.967±	0.9309
	F-75 <sub>E</sub>	5.717±	0.9559	M-75 <sub>E</sub>	5.983±	1.0572
	F-5 <sub>G</sub>	4.850±	0.8408	M-5 <sub>G</sub>	4.850±	0.3209
	F-50 <sub>G</sub>	4.900±	0.6573	M-50 <sub>G</sub>	4.583±	0.7333
	F-75 <sub>G</sub>	5.167±	1.2453	M-75 <sub>G</sub>	5.750±	1.1059
	F-Control	5.300±	1.0234	M-Control	5.792±	1.1341
<b>Lym</b> %	F-5 <sub>E</sub>	3.400±	0.5292	M-5 <sub>E</sub>	3.950±	0.9670
	F-50 <sub>E</sub>	4.250±	0.6091	M-50 <sub>E</sub>	4.250±	0.8550
	F-75 <sub>E</sub>	4.817±	0.5845	M-75 <sub>E</sub>	5.283±	0.9968
	F-5 <sub>G</sub>	4.117±	0.8424	M-5 <sub>G</sub>	4.100±	0.2898
	F-50 <sub>G</sub>	4.300±	0.4690	M-50 <sub>G</sub>	4.033±	0.5086
	F-75 <sub>G</sub>	3.900±	0.8222	M-75 <sub>G</sub>	5.083±	0.9261
	F-Control	4.567±	0.8139	M-Control	4.900±	1.0445
<b>Mon</b> %	F-5 <sub>E</sub>	0.333±	0.1633	M-5 <sub>E</sub>	0.350±	0.1225
	F-50 <sub>E</sub>	0.533±	0.0816	M-50 <sub>E</sub>	0.433±	0.1506
	F-75 <sub>E</sub>	0.683±	0.4262	M-75 <sub>E</sub>	0.600±	0.2098
	F-5 <sub>G</sub>	0.517±	0.1472	M-5 <sub>G</sub>	0.517±	0.0983
	F-50 <sub>G</sub>	0.650±	0.3782	M-50 <sub>G</sub>	0.233±	0.1033
	F-75 <sub>G</sub>	0.717±	0.7960	M-75 <sub>G</sub>	0.500±	0.2366
	F-Control	0.517±	0.2038	M-Control	0.600±	0.1477

Table 5. Effect of Lectranal® on haematological parameters

BIOCHEMICAL PARAMETERS						
parameter	female			male		
	group	Mean±SD		group	Mean±SD	
<b>Proteins</b> g/L	F-5 <sub>E</sub>	59.00±	2.757	M-5 <sub>E</sub>	59.17±	2.137
	F-50 <sub>E</sub>	56.67±	2.338	M-50 <sub>E</sub>	60.17±	1.602
	F-75 <sub>E</sub>	61.00±	2.098	M-75 <sub>E</sub>	61.50±	1.871
	F-5 <sub>G</sub>	60.00±	1.414	M-5 <sub>G</sub>	58.00±	0.632
	F-50 <sub>G</sub>	58.83±	2.787	M-50 <sub>G</sub>	57.50±	2.168
	F-75 <sub>G</sub>	58.50±	2.588	M-75 <sub>G</sub>	59.83±	0.753
	F-Control	57.83±	3.486	M-Control	58.08±	2.151
<b>Albumin</b> g/L	F-5 <sub>E</sub>	34.483±	0.4622	M-5 <sub>E</sub>	33.183±	0.4622
	F-50 <sub>E</sub>	33.567±	0.6563	M-50 <sub>E</sub>	33.517±	1.1125
	F-75 <sub>E</sub>	34.117±	0.2994	M-75 <sub>E</sub>	33.650±	1.0330
	F-5 <sub>G</sub>	33.717±	1.3378	M-5 <sub>G</sub>	32.567±	0.9688
	F-50 <sub>G</sub>	33.333±	1.7818	M-50 <sub>G</sub>	31.667±	1.5082
	F-75 <sub>G</sub>	33.467±	0.5428	M-75 <sub>G</sub>	33.850±	0.6442
	F-Control	33.058±	1.9519	M-Control	32.517±	1.2590
<b>Glucose</b> mmol/L	F-5 <sub>E</sub>	3.600±	1.2791	M-5 <sub>E</sub>	4.850±	1.7248
	F-50 <sub>E</sub>	3.500±	1.1950	M-50 <sub>E</sub>	5.667±	1.1165
	F-75 <sub>E</sub>	4.483±	0.6646	M-75 <sub>E</sub>	4.217±	1.2891
	F-5 <sub>G</sub>	4.250±	1.1845	M-5 <sub>G</sub>	4.583±	0.5707
	F-50 <sub>G</sub>	4.983±	1.7623	M-50 <sub>G</sub>	6.867±	0.9791
	F-75 <sub>G</sub>	4.200±	0.5692	M-75 <sub>G</sub>	4.233±	0.7916
	F-Control	4.408±	0.4889	M-Control	4.358±	1.0326
<b>Cholesterol</b> mmol/L	F-5 <sub>E</sub>	3.4833±	0.3158	M-5 <sub>E</sub>	3.7283±	0.1893
	F-50 <sub>E</sub>	3.1517±	0.1156	M-50 <sub>E</sub>	3.7767±	0.1440
	F-75 <sub>E</sub>	3.4183±	0.1967	M-75 <sub>E</sub>	3.9617±	0.2074
	F-5 <sub>G</sub>	3.1983±	0.5312	M-5 <sub>G</sub>	3.6783±	0.0895
	F-50 <sub>G</sub>	3.0367±	0.1202	M-50 <sub>G</sub>	3.4183±	0.6521
	F-75 <sub>G</sub>	3.1350±	0.2627	M-75 <sub>G</sub>	3.9600±	0.3283
	F-Control	3.0475±	0.3819	M-Control	3.4000±	0.4630

Table 6. Effect of Lectranal® on biochemical parameters

Biochemical parameters						
parameter	female			male		
	group	Mean±SD		group	Mean±SD	
Urea mmol/L	F-5 <sub>E</sub>	6.717±	0.4119	M-5 <sub>E</sub>	5.617±	0.4401
	F-50 <sub>E</sub>	7.067±	1.5332	M-50 <sub>E</sub>	7.100±	1.3431
	F-75 <sub>E</sub>	9.917±	0.6585	M-75 <sub>E</sub>	7.533±	0.4227
	F-5 <sub>G</sub>	6.133±	0.4803	M-5 <sub>G</sub>	7.050±	0.7287
	F-50 <sub>G</sub>	6.083±	1.7081	M-50 <sub>G</sub>	6.233±	0.5502
	F-75 <sub>G</sub>	7.317±	0.8085	M-75 <sub>G</sub>	6.217±	0.2483
	F-Control	6.333±	1.3096	M-Control	6.442±	0.4379
Creatinine µmol/L	F-5 <sub>E</sub>	43.33±	2.805	M-5 <sub>E</sub>	38.33±	2.582
	F-50 <sub>E</sub>	46.33±	3.266	M-50 <sub>E</sub>	42.17±	2.317
	F-75 <sub>E</sub>	51.50±	8.961	M-75 <sub>E</sub>	39.83±	2.041
	F-5 <sub>G</sub>	43.67±	1.211	M-5 <sub>G</sub>	40.33±	2.160
	F-50 <sub>G</sub>	43.67±	4.131	M-50 <sub>G</sub>	39.83±	1.722
	F-75 <sub>G</sub>	46.50±	4.037	M-75 <sub>G</sub>	40.33±	3.327
	F-Control	46.33±	10.210	M-Control	41.25±	2.179
AST IU/L	F-5 <sub>E</sub>	125.00±	22.208	M-5 <sub>E</sub>	78.50±	16.670
	F-50 <sub>E</sub>	99.17±	18.509	M-50 <sub>E</sub>	83.00±	31.718
	F-75 <sub>E</sub>	125.67±	38.847	M-75 <sub>E</sub>	121.83±	31.199
	F-5 <sub>G</sub>	111.33±	22.447	M-5 <sub>G</sub>	66.83±	9.517
	F-50 <sub>G</sub>	100.50±	26.674	M-50 <sub>G</sub>	69.00±	32.918
	F-75 <sub>G</sub>	111.33±	28.472	M-75 <sub>G</sub>	101.67±	21.472
	F-Control	111.08±	22.649	M-Control	106.58±	27.734
Calcium mmol/L	F-5 <sub>E</sub>	2.3383±	0.14932	M-5 <sub>E</sub>	2.3000±	0.08556
	F-50 <sub>E</sub>	2.4267±	0.07891	M-50 <sub>E</sub>	2.3950±	0.09649
	F-75 <sub>E</sub>	2.3633±	0.18576	M-75 <sub>E</sub>	2.2083±	0.17046
	F-5 <sub>G</sub>	2.3717±	0.12952	M-5 <sub>G</sub>	2.3150±	0.03017
	F-50 <sub>G</sub>	2.4550±	0.09524	M-50 <sub>G</sub>	2.3383±	0.08704
	F-75 <sub>G</sub>	2.3067±	0.06532	M-75 <sub>G</sub>	2.3350±	0.10521
	F-Control	2.4442±	0.12369	M-Control	2.2925±	0.05225
Potassium mmol/L	F-5 <sub>E</sub>	10.783±	0.9786	M-5 <sub>E</sub>	9.850±	0.6834
	F-50 <sub>E</sub>	10.550±	0.6156	M-50 <sub>E</sub>	9.433±	1.0985
	F-75 <sub>E</sub>	10.100±	0.3098	M-75 <sub>E</sub>	9.700±	1.6297
	F-5 <sub>G</sub>	10.533±	0.7090	M-5 <sub>G</sub>	9.617±	0.7521
	F-50 <sub>G</sub>	10.117±	0.3251	M-50 <sub>G</sub>	9.800±	1.0881
	F-75 <sub>G</sub>	9.717±	0.4622	M-75 <sub>G</sub>	10.500±	0.8246
	F-Control	10.783±	0.6939	M-Control	10.458±	1.2391

Table 7. Effect of Lectranal® on biochemical parameters

### Effects of Lectranal® on gross necropsy

All animals in the study were subjected to detailed gross necropsy. The surfaces of the body, all orifices, and the cranial, thoracic, and abdominal cavities were examined. The liver, kidneys, adrenals, brain, thymus, spleen, testes/ovaries, and heart were carefully examined and weighed wet. No changes were revealed by necropsy and there was no difference in organ weight compared to the control animals. Organ weights are shown in Table 8 and Table 9.

ORGAN WEIGHT						
parameter	female			male		
	group	Mean (g) ±SD		group	Mean (g) ±SD	
Liver	F-5 <sub>E</sub>	1058.83±	122.943	M-5 <sub>E</sub>	1094.50±	27.186
	F-50 <sub>E</sub>	1046.33±	62.800	M-50 <sub>E</sub>	1323.67±	172.125
	F-75 <sub>E</sub>	1142.17±	101.170	M-75 <sub>E</sub>	1456.83±	101.263
	F-5 <sub>G</sub>	0998.50±	70.136	M-5 <sub>G</sub>	1174.33±	172.175
	F-50 <sub>G</sub>	1190.00±	394.562	M-50 <sub>G</sub>	1244.67±	120.858
	F-75 <sub>G</sub>	1027.00±	296.070	M-75 <sub>G</sub>	1164.33±	77.518
	F-Control	1035.75±	99.108	M-Control	1123.42±	115.313

<b>Kidney</b>	F-5 <sub>E</sub>	143.67±	13.765	M-5 <sub>E</sub>	199.00±	29.846
	F-50 <sub>E</sub>	145.83±	22.675	M-50 <sub>E</sub>	231.83±	20.272
	F-75 <sub>E</sub>	147.83±	10.088	M-75 <sub>E</sub>	253.00±	28.093
	F-5 <sub>G</sub>	148.17±	23.413	M-5 <sub>G</sub>	212.50±	26.980
	F-50 <sub>G</sub>	132.50±	15.783	M-50 <sub>G</sub>	207.83±	28.372
	F-75 <sub>G</sub>	138.17±	17.116	M-75 <sub>G</sub>	214.67±	21.248
	F-Control	153.58±	8.554	M-Control	241.50±	23.524
<b>Adrenal gland</b>	F-5 <sub>E</sub>	4.17±	1.722	M-5 <sub>E</sub>	4.33±	1.751
	F-50 <sub>E</sub>	4.50±	1.871	M-50 <sub>E</sub>	4.00±	1.549
	F-75 <sub>E</sub>	5.00±	2.530	M-75 <sub>E</sub>	3.50±	1.751
	F-5 <sub>G</sub>	4.83±	3.189	M-5 <sub>G</sub>	5.33±	2.658
	F-50 <sub>G</sub>	4.50±	2.168	M-50 <sub>G</sub>	4.00±	2.608
	F-75 <sub>G</sub>	3.33±	1.633	M-75 <sub>G</sub>	2.17±	0.753
	F-Control	5.00±	1.954	M-Control	3.92±	3.476
<b>Brain</b>	F-5 <sub>E</sub>	393.00±	59.090	M-5 <sub>E</sub>	387.83±	22.649
	F-50 <sub>E</sub>	384.00±	47.248	M-50 <sub>E</sub>	360.33±	44.680
	F-75 <sub>E</sub>	410.17±	25.199	M-75 <sub>E</sub>	420.00±	42.341
	F-5 <sub>G</sub>	420.33±	24.180	M-5 <sub>G</sub>	388.17±	42.258
	F-50 <sub>G</sub>	376.17±	31.492	M-50 <sub>G</sub>	366.50±	23.688
	F-75 <sub>G</sub>	384.83±	28.231	M-75 <sub>G</sub>	393.50±	31.079
	F-Control	403.33±	38.785	M-Control	398.58±	34.305

Table 8. Effect of Lectranal® on wet organ weight

ORGAN WEIGHT						
parameter	female			male		
	group	Mean (g) ±SD		group	Mean (g) ±SD	
<b>Thymus</b>	F-5 <sub>E</sub>	31.33±	9.114	M-5 <sub>E</sub>	33.00±	16.087
	F-50 <sub>E</sub>	33.33±	8.238	M-50 <sub>E</sub>	33.50±	16.778
	F-75 <sub>E</sub>	36.83±	14.689	M-75 <sub>E</sub>	33.17±	12.937
	F-5 <sub>G</sub>	39.50±	15.604	M-5 <sub>G</sub>	33.17±	10.206
	F-50 <sub>G</sub>	31.67±	11.827	M-50 <sub>G</sub>	40.17±	16.290
	F-75 <sub>G</sub>	28.67±	11.130	M-75 <sub>G</sub>	28.50±	8.503
	F-Control	32.67±	5.726	M-Control	24.58±	8.806
<b>Spleen</b>	F-5 <sub>E</sub>	59.00±	5.099	M-5 <sub>E</sub>	60.83±	7.757
	F-50 <sub>E</sub>	57.17±	7.111	M-50 <sub>E</sub>	78.50±	15.398
	F-75 <sub>E</sub>	58.67±	9.004	M-75 <sub>E</sub>	62.83±	10.962
	F-5 <sub>G</sub>	64.83±	16.130	M-5 <sub>G</sub>	56.33±	3.327
	F-50 <sub>G</sub>	78.00±	14.427	M-50 <sub>G</sub>	64.83±	5.115
	F-75 <sub>G</sub>	58.67±	5.854	M-75 <sub>G</sub>	64.33±	14.855
	F-Control	63.33±	11.420	M-Control	67.00±	17.560
<b>Testicle/ Ovary</b>	F-5 <sub>E</sub>	9.00±	2.608	M-5 <sub>E</sub>	68.67±	15.744
	F-50 <sub>E</sub>	12.83±	2.401	M-50 <sub>E</sub>	69.00±	6.132
	F-75 <sub>E</sub>	8.17±	1.472	M-75 <sub>E</sub>	60.33±	7.659
	F-5 <sub>G</sub>	10.83±	3.545	M-5 <sub>G</sub>	63.50±	5.244
	F-50 <sub>G</sub>	10.50±	4.416	M-50 <sub>G</sub>	62.50±	10.821
	F-75 <sub>G</sub>	20.33±	24.921	M-75 <sub>G</sub>	64.50±	6.189
	F-Control	7.67±	4.228	M-Control	59.25±	7.921
<b>Heart</b>	F-5 <sub>E</sub>	109.00±	11.082	M-5 <sub>E</sub>	132.50±	15.320
	F-50 <sub>E</sub>	105.00±	11.524	M-50 <sub>E</sub>	124.00±	10.936
	F-75 <sub>E</sub>	111.17±	16.425	M-75 <sub>E</sub>	145.33±	18.118
	F-5 <sub>G</sub>	108.50±	9.915	M-5 <sub>G</sub>	127.50±	18.447
	F-50 <sub>G</sub>	97.83±	13.378	M-50 <sub>G</sub>	126.83±	13.438
	F-75 <sub>G</sub>	98.17±	10.722	M-75 <sub>G</sub>	123.17±	7.985
	F-Control	106.75±	9.706	M-Control	131.58±	15.559

Table 9. Effect of Lectranal® on wet organ weight

**Effects of Lectranal® on histopathology**

Histopathological analysis was carried out on the brain, stomach, intestine, adrenal gland, liver, kidneys, spleen, heart, thymus, thyroid gland, lungs, testicles/ovaries, urinary bladder, and lymph nodes of each animal in the control and high dose (75 mg) Lectranal® groups.

Analysis by electronic microscope showed that all sections have regular structure with no lesions, apoptosis, infiltration or other pathological changes. Inhalatory pneumonia was found in two animals. This finding is a result of a technical error during the pipe insertion and is not attributed to the treatment.

**CONCLUSION**

Oral administration of Lectranal® to mice during 28 days did not cause any changes that might be considered the result of the experiment. Lectranal® caused no toxicity effects of any kind.