Food and Water Consumption in Assessment of Acute Oral Toxicity of Hepalip Forte¨ in Rats

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Abstract

Body weight variations during toxicological testing can be one of the indicators of the test substance toxic effects. Data on food and water consumption are true indicators of the rate of growth of experimental animals (Stevens & Gallo, 1989). Daily recording of the food and water consumption was done during the acute toxicity testing of **HEPALIP FORTE®**. The study was performed on Wistar rats. The active component of **HEPALIP FORTE®** is EPL substance - essential phospholipids, a natural substance present in every living cell. Essential phospholipids in combination with vitamins have been used in the treatment of liver diseases, dyslipoproteinaemias and intoxications accompanied with liver failure.

Statistical analysis of the body weight variations was performed separately, for males and females. The analysis failed to show any significant difference between the groups. There was a significant difference in water consumption between the male group 2M and female groups 3F and 2F in comparison with control groups. Statistical analysis of the variations of food consumption showed a significant difference in all male groups in comparison with control groups, and only in the 3F female group in comparison with a control group.

Considering the absence of lethality and the lack of significant influence of the test substance on animal body weights, we concluded that the test substance was not acutely toxic in rats, if applied orally, in single doses of 300 mg/kg, 500 mg/kg and 1000 mg/kg. Significant differences found in food and water consumption suggest a need of their during the future chronic toxicity testing.

Key words: acute toxicity, food and water consumption, essential phospholipids.

Introduction

Polyen-phosphatidyl-cholin (PPC) known as essential phospholipids (EPL) is a natural substance present in every living cell: herbal, animal or human. Their chemical structure corresponds to endogen phospholipids, but they have functional superiority because of the content of unsaturated fatty acids. Essential phospholipids integrate in the cell membrane and organelle systems and become constitutive elements of the cell membranes.

Main effects of EPL are related to the following: they are incorporated in the structure of lipoprotein complexes (Zulu et al., 1996), they have metabolic (Zulu et al., 1982), homodynamic (Zulu et al., 1989) and hepatic effects, and they positively influence the longitude of animal life duration and male fertility. Essential phospholipids in combination with vitamins have been used in the treatment of liver diseases, dyslipoproteinaemias and intoxications accompanied with liver failure.

Body weight variations during the toxicological testing can be one of the indicators of the test substance toxic effects. Data on food and water consumption are true indicators of the rate of growth of experimental animals (Stevens & Gallo, 1989). A recording of these parameters is common in chronic toxicity testing, in order to determine the treatment effects on appetite and usage of the ingested food. Weekly records of body weight variations may indicate disease or debility in older experimental animals. This data can be a key factor in the determination of toxicological significance of the results. The aim of this study is to show that data about the food and water consumption can be valuable indicators in acute toxicity studies, as well.

Material and methods

Daily recording of the food and water consumption was done during the acute toxicity testing of **HEPALIP FORTE®**. The study was performed on the albino Wistar rats. During the randomisation, all animals with unsatisfying body weight or health condition were excluded. Animals were randomised in 3 experimental and 1 control group, 5 males and 5 females in each. Animals were in quarantine for 2 weeks. During that observation period, animal health condition was examined while all eventual symptoms were recorded. Food and water consumption were recorded every morning. Determination of the each animal body weight was done before the test substance application, after 7 days and at the end of the study.
Study included one oral dose of the test substance,

Table 1. Body weight before application of test substance

No	Group	No of animals	Group body weight mean/g	STDEV	Total group body weight/g
1	3M	5	434.2	35.25	2171
2	2M	5	436	27.91	2180
3	1M	5	443.8	12.48	2219
4	KM	5	450.2	15.02	2251
5	3F	5	281.2	15.71	1406
6	2F	5	301	29.64	1505
7	1F	5	320.2	27.88	1601
8	KF	5	296.2	37.56	1481

Table 2. Body weight before sacrifice

No	Group	No of animals	Group body weight mean/g	STDEV	Total group body weight/g	Difference between total group body weights/g
1	3M	5	422.6	25.32	2113	-42
2	2M	5	415.6	30.14	2078	-102
3	1M	5	430.4	15.5	2152	-67
4	KM	5	429.8	14.86	2149	-2
5	3F	5	278.8	17.12	1394	-12
6	2F	5	293.6	22.1	1468	-37
7	1F	5	314.8	23.42	1574	-27
8	KF	5	287.4	36.14	1437	-44

applied by oesophageal intubations. Test substance application day was the first day of the observation period that lasted 14 days. There were 3 dose-levels: 300, 500 and 1000 mg/kg. Control animals were treated with the vehicle (milk). Dose concentrations of the test substance were prepared just before the application.

The test samples were HEPALIP FORTE® capsules, manufactured by Bosnalijek d.d. Sarajevo. The content of capsules was dissolved in milk heated in the water bath up to 60°C. Composition of the each capsule is:

- Active components: EPL substance 300 mg, Nicotinamide 30 mg, Pyridoxine hydrochloride 6 mg, Riboflavin 6 mg, Thiamine nitrate 6 mg, Tocopherol acetate 6 mg;
- Other constitutes: *triglycerida saturata media*, hard gelatinous capsule N°1.

Results

There was no lethality during the study. Daily food and water consumption and weekly body weight variations were recorded.

Statistical analysis was done in the Microsoft® Excel 2002 and SigmaStat for Windows 2.03 programs. Experimental animals were randomised in 8 groups (4 male and 4 female groups). Groups were similar according to their characteristics and consisted of five animals each. In the text that follows, all animal groups will be coded because of the easier result presentation. Animals in the groups 1, 2 and 3 were treated with following single doses: "1" - 300 mg/kg, "2" - 500 mg/kg and "3" - 1000 mg/kg. "K" letter is a sign for the control animals.

Experimental animals were randomised, so the mean body weight did not significantly differ between groups ($p=0.5$). Variations inside experimental groups were within two standard deviations. Animal body weights before the test substance application and before the planned sacrifice and necropsy are presented in Tables 1 and 2.

Statistical analysis of the body-weight variations was performed separately for males and females. T-test for the independent specimen failed to show any significant difference between the groups. These results are presented in Tables 3 and 4.

A statistical analysis of the variations of water consumption was done throughout the descriptive statistics and the T-test for independent specimen. These records are presented in Table 5 while descriptive statistics in Table 6.

Differences in the water consumption between the groups were analysed throughout the T-test for independent specimen. There was a significant difference in the water consumption between the male group 2M and the control

Table 3. Differences in mean body weights between male groups

Difference for the groups	N	Mean	Std Dev	SEM
3M	5	11.600	12.442	5.564
KM	5	20.400	8.355	3.736
Difference: -8.800 t = -1.313 with 8 degrees of freedom (P = 0.226)				
Difference for the groups	**N**	**Mean**	**Std Dev**	**SEM**
2M	5	20.400	6.914	3.092
KM	5	20.400	8.355	3.736
Difference: 0.000 t = 0.000 with 8 degrees of freedom (P = 1.000)				
Difference for the groups	**N**	**Mean**	**Std Dev**	**SEM**
1M	5	13.400	5.639	2.522
KM	5	20.400	8.355	3.736
Difference: -7.000 t = -1.553 with 8 degrees of freedom (P = 0.159)				

Table 4. Differences in mean body weights between female groups

Difference for the groups	N	Mean	Std Dev	SEM
3F	5	2.400	6.427	2.874
KF	5	8.800	3.271	1.463
Difference: -6.400 t = -1.985 with 8 degrees of freedom (P = 0.082)				
Difference for the groups	**N**	**Mean**	**Std Dev**	**SEM**
2F	5	7.400	7.668	3.429
KF	5	8.800	3.271	1.463
Difference: -1.400 t = -0.376 with 8 degrees of freedom. (P = 0.717)				
Difference for the groups	**N**	**Mean**	**Std Dev**	**SEM**
1F	5	5.400	10.922	4.885
KF	5	8.800	3.271	1.463
Difference: -3.400 t = -0.667 with 8 degrees of freedom. (P = 0.524)				

group. In female groups, a statistically significant difference was found in groups 3F (p 0.001) and 2F (p=0.002) in comparison with control groups. These results are presented in Tables 7 and 8.

A statistical analysis of the variations of food consumption was done throughout the descriptive statistics and the T-test for independent specimen. These records are presented in Table 9 while descriptive statistics in Table 10.

Differences in the food consumption between the groups were analysed throughout the T-test for independent specimen. There was a significant difference in the food consumption in all male groups in comparison with control groups. In female groups, a statistically significant difference was only found in the 3F group in comparison with a control group. These results are presented in Tables 11 and 12.

Discussion

During this study, there was no lethality. We concluded that the test substance applied in rationally high doses demonstrated a low level of toxicity. Body weight variations can be considered as sensitive, but non-specific indicators of animal health condition (Gad & Changelis, 1998), especially in subchronic and chronic toxicity studies. There are some reserves about the value of this parameter as valid indicator in acute toxicity studies being performed with very high doses of the test substance. Food consumption can indicate the hidden reasons for the body weight variations. Body weight lost and normal food consumption is a very different thing from the body weight lost as a result of the decreased food consumption.

Comparison of the animal body weights at the beginning and at the end of the study suggests a mild body weight lost. This fall is not greater than 10% of the animal mean body weight and can also be observed in control groups

Table 5. Water consumption recordings

Consumption								
Day	Group 3M	Group 2M	Group 1M	Control M	Group 3F	Group 2F	Group 1F	Control F
1(21.02.03)	210	210	250	280	150	175	200	200
2 (22.02.03)	180	200	200	200	150	180	180	120
Differences	30	10	50	80	0	-5	20	80
3(23.02.03)	175	180	195	200	145	130	150	160
Differences	5	20	5	0	5	50	30	-40
4(24.02.03.)	220	205	225	200	150	175	175	225
Differences	-45	-25	-30	0	-5	-45	-25	-65
5(25.02.03.)	225	200	200	225	150	160	175	180
Differences	-5	5	25	-25	0	15	0	45
6(26.02.03.)	220	200	200	200	130	150	195	190
Differences	5	0	0	25	20	10	-20	-10
7(27.02.03.)	205	180	195	210	135	160	180	195
Differences	15	20	5	-10	-5	-10	15	-5
8(28.02.03.)	195	190	200	200	130	175	175	180
Differences	10	-10	-5	10	5	-15	5	15
9(01.03.03.)	200	175	160	185	140	150	155	175
Differences	-5	15	40	15	-10	25	20	5
10(02.03.03.)	200	175	200	200	150	175	190	200
Differences	0	0	-40	-15	-10	-25	-35	-25
11(03.03.03.)	210	180	225	200	150	150	175	180
Differences	-10	-5	-25	0	0	25	15	20
12(04.03.03.)	190	200	225	225	225	125	160	190
Differences	20	-20	0	-25	-75	25	15	-10
13(05.03.03.)	222	150	200	200	150	160	150	200
Differences	-32	50	25	25	75	-35	10	-10
14(06.03.03.)	220	215	250	250	160	160	225	210
Differences	2	-65	-50	-50	-10	0	-75	-10
Mean	205.1429	190	208.9286	**212.5**	151.0714	158.9286	177.5	**186.07**

Table 6. Water consumption descriptive statistics

Group	No of recordings	Mean	Std Dev	Std. Error	C.I. of Mean
3M	14	205.143	15.966	4.267	9.218
2M	14	190.000	17.541	4.688	10.128
1M	14	208.929	23.873	6.380	13.784
KM	14	212.500	25.249	6.748	14.578
3F	14	151.071	22.970	6.139	13.262
2F	14	158.929	16.891	4.514	9.753
1F	14	177.500	20.732	5.541	11.970
KF	14	186.071	24.898	6.654	14.376

of animals. Body weight lost is not presumed to be result of the test substance toxicity.

Combination of these results with mild fall in the food consumption after dosing can be used for the negation of substances catabolic toxic effects. The fall in consumption was also seen in the controls that are dosed with milk and can be the consequence of application the large amount of substance and vehicle. The fall in water consumption can be seen in females treated with the highest dose on the first day. In this group the most frequently recorded clinical sign in the study (tremor) was more present than in other groups.

A statistically significant difference between experimental animal groups and control group of animals can be noticed after the comparison of all 14 food consumption measurements with exclusion of the food consumption in

Table 7. Differences in water consumption between male groups

Difference for groups	N	Mean	Std Dev	SEM
3M	14	205.143	15.966	4.267
KM	14	212.500	25.249	6.748
Difference: -7.357 t = -0.921 with 26 degrees of freedom (P = 0.365) Difference is not statistically significant P>0.05				
Difference for groups	**N**	**Mean**	**Std Dev**	**SEM**
2M	14	190.000	17.541	4.688
KM	14	212.500	25.249	6.748
Difference -22.500 t = -2.738 with 26 degrees of freedom (P = 0.011) Difference is statistically significant P<0.05				
Difference for groups	**N**	**Mean**	**Std Dev**	**SEM**
1M	14	208.929	23.873	6.380
KM	14	212.500	25.249	6.748
Difference -3.571 t = -0.385 with 26 degrees of freedom (P = 0.704) Difference is not statistically significant P>0.05				

Table 8. Differences in water consumption between female groups

Difference for groups	N	Mean	Std Dev	SEM
3F	14	151.071	22.970	6.139
KF	14	186.071	24.898	6.654
Difference: -35.000 t = -3.866 with 26 degrees of freedom (P = <0.001) Difference is statistically significant P<0.001				
Difference for groups	**N**	**Mean**	**Std Dev**	**SEM**
2F	14	158.929	16.891	4.514
KF	14	186.071	24.898	6.654
Difference: --27.143 t = -3.376 with 26 degrees of freedom (P = 0.002) Difference is statistically significant P=0.002				
Difference for groups	**N**	**Mean**	**Std Dev**	**SEM**
1F	14	177.500	20.732	5.541
KF	14	186.071	24.898	6.654
Difference: --8.571 t = -0.990 with 26 degrees of freedom (P = 0.331) Difference is not statistically significant. P>0.05				

the 1F group. Considering the lack of statistically significant differences in the group body weight variations, all mentioned data might suggest the influence of the test substance application on the appetite or indicate the metabolic pharmacodynamic effects.

Conclusion

After statistical analysis of data (descriptive statistics and T-test for independent specimens) on acute toxicity testing we can conclude the following:

1. Body weight: the test substance did not induce statistically significant changes in the animal body weights when comparing experimental and control animals.
2. Food consumption: test substance induced statistically significant changes in the food consumption in groups of animals 3M, 2M, 1M and 3F in comparison with control group of animals.
3. Water consumption: the test substance induced statistically significant changes in the water consumption in groups of animals 3M, 3F and 2F in comparison with control group of animals.

Considering the absence of lethality and the lack of significant influence of the test substance on animal body weights, we can conclude that the test substance was not acutely toxic when applied orally in rats, in single doses of 300 mg/kg, 500 mg/kg and 1000 mg/kg. Significant differences in food and water consumption suggest a need of the monitoring of these parameters during the chronic toxicity testing.

Table 9. Food consumption recordings

Consumption								
Day	Group 3M	Group 2M	Group 1M	Control M	Group 3F	Group 2F	Group 1F	Control F
1(21.02.03)	123.75	121.5	126	130.5	95.45	96.75	99	94.5
2 (22.02.03)	99	117	121.5	126	76.5	85.5	101.25	81
Differences	24.75	4.5	4.5	4.5	18.95	11.25	-2.25	13.5
3(23.02.03)	117	110.25	126	126	72	85.5	103.5	99
Differences	-18	6.75	-4.5	0	4.5	0	-2.25	-18
4(24.02.03.)	117	126	126	126	85.5	94.5	103.5	108
Differences	0	-15.75	0	0	-13.5	-9	0	-9
5(25.02.03.)	121.5	126	126	126	85.5	99	99	99
Differences	-4.5	0	0	0	0	-4.5	4.5	9
6(26.02.03.)	119.25	126	123.75	132.75	81	94.5	114.75	94.5
Differences	2.25	0	2.25	-6.75	4.5	4.5	-15.75	4.5
7(27.02.03.)	130.5	130.5	126	134.75	94.5	103.5	103.5	99
Differences	-11.25	-4.5	-2.25	-2	-13.5	-9	11.25	-4.5
8(28.02.03.)	112.5	117	128.25	130.5	81	99	94.5	112.5
Differences	18	13.5	-2.25	4.25	13.5	4.5	9	-13.5
9(01.03.03.)	126	128.25	121.5	130.5	85.5	99	103.5	85.5
Differences	-13.5	-11.25	6.75	0	-4.5	0	-9	27
10(02.03.03.)	121.5	117	126	135	81	96.75	126	96.75
Differences	4.5	11.25	-4.5	-4.5	4.5	2.25	-22.5	-11.25
11(03.03.03.)	126	121.5	121.5	135	81	90	90	103.5
Differences	-4.5	-4.5	4.5	0	0	6.75	36	-6.75
12(04.03.03.)	117	126	121.5	126	85.5	85.5	85.5	108
Differences	9	-4.5	0	9	-4.5	4.5	4.5	-4.5
13(05.03.03.)	115	126	108	135	85.5	85.5	96.75	103.5
Differences	2	0	13.5	-9	0	0	-11.25	4.5
14(06.03.03.)	121.5	126	135	130.5	81	90	117	94.5
Differences	-6.5	0	-27	4.5	4.5	-4.5	-20.25	9
Mean	119.1071	122.7857	124.0714	**130.32**	90.78214	93.21429	102.6964	**98.518**

Table 10. Food consumption descriptive statistics

Group	No of recordings	Mean	Std Dev	Std. Error	C.I. of Mean
3M	14	119.571	7.481	1.999	4.319
2M	14	122.786	5.631	1.505	3.251
1M	14	124.071	5.844	1.562	3.375
KM	14	130.321	3.769	1.007	2.176
3F	14	83.639	6.177	1.651	3.567
2F	14	93.214	6.160	1.646	3.556
1F	14	102.696	10.686	2.856	6.170
KF	14	98.518	8.564	2.289	4.945

Table 11. Differences in food consumption between male groups

Difference for groups	N	Mean	Std Dev	SEM
3M	14	119.571	7.481	1.999
KM	14	130.321	3.769	1.007
Difference: --10.750 t = -4.802 with 26 degrees of freedom (P = <0.001) Difference is statistically significant P<0.001				
Difference for groups	**N**	**Mean**	**Std Dev**	**SEM**
2M	14	122.786	5.631	1.505
KM	14	130.321	3.769	1.007
Difference: --7.536 t = -4.161 with 26 degrees of freedom (P = <0.001) Difference is statistically significant P<0.001				
Difference for groups	**N**	**Mean**	**Std Dev**	**SEM**
1M	14	124.071	5.844	1.562
KM	14	130.321	3.769	1.007
Difference: -6.250 t = -3.363 with 26 degrees of freedom (P = 0.002) Difference is statistically significant P=0.002				

Table 12. Differences in food consumption between female groups

Difference for groups	N	Mean	Std Dev	SEM
3F	14	83.639	6.177	1.651
KF	14	98.518	8.564	2.289
Difference: --14.879 t = -5.272 with 26 degrees of freedom. (P = <0.001) Difference is statistically significant. P<0.001.				
Difference for groups	**N**	**Mean**	**Std Dev**	**SEM**
2F	14	93.214	6.160	1.646
KF	14	98.518	8.564	2.289
Difference: --5.304 T = -1.881 with 26 degrees of freedom. (P = 0.071) Difference is not statistically significant. P>0.05.				
Difference for groups	**N**	**Mean**	**Std Dev**	**SEM**
1F	14	102.696	10.686	2.856
KF	14	98.518	8.564	2.289
Difference: --4.179 t = 1.142 with 26 degrees of freedom. (P = 0.264) Difference is not statistically significant. P>0.05.				

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