

R4

**The study of Bio-normalizer (BN) clinical efficacy,
free radical- and immunomodulating activity in the
treatment of insulin-dependent diabetes mellitus**

Korkina LG, et. al

TRIAL PROTOCOL

(confidential)

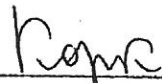
Pilot Clinical Trial (Phase II)

Title: THE STUDY OF BIO-NORMALIZER (BN) CLINICAL EFFICACY AND IMMUNOMODULATING ACTIVITY IN THE TREATMENT OF INSULIN-DEPENDENT DIABETES MELLITUS

Professor Michail I. Balabolkin
Director, Institute of Diabetes

Signature

Professor Ludmila G. Korkina, M.D., Ph.D., Dr.Sci.
Research Supervisor



Signature

Dr. Ashot M. Mkrtumyan, M.D.
Clinical Investigator

Signature

Table 1 The effect of BN administration on monocyte luminol-amplified chemiluminescence

N	Spont 2nd visit	Spont 3d visit	PMA -activ. 2nd visit	PMA -activ. 3d visit	PMA+ NAME 2nd visit	PMA+ NAME 3d visit	Zym- activ. 2nd visit	Zym- activ. 3d visit	Zym+ NAME 2nd visit	Zym+ NAME 3d visit
1	9	261	54	384	9	223	8	4240	8	2650
2	27	130	157	103	36	49	73	303	72	265
3	74	578	1024	625	354	308	803	2820	715	1914
4	60	687	572	463	380	272	590	5192	425	4143
5	83	n.a.*	154	n.a.*	57	n.a.*	113	n.a.*	118	n.a.*
6	34	48	30	356	14	146	10	1616	8	952
7	232	75	876	262	72	117	310	2522	165	1230
8	62	51	870	301	187	232	4	1286	3	1005
9	10	392	8	497	5	275	5	4424	2	1825
10	18	197	8	5520	3	1800	0	4100	0	2538
11	21	51	35	301	18	18	27	1188	20	706
12	31	60	51	382	15	179	5	2588	2	1516
13	93	288	540	559	301	326	1729	7254	1388	2866
14	180	200	963	1068	303	652	1468	4518	923	1965
15	500	394	1483	894	720	421	2767	4780	1640	2529

Table 2 Effect of BN administration on monocyte lucigenin-amplified CL

N	Spont 2nd visit	Spont 3d visit	PMA-activ. 2nd visit	PMA-activ. 3d visit	Zym-activ 2nd visit	Zym-activ. 3d visit
1	1	2	9	40	5	120
2	4	3	23	52	18	312
3	11	7	119	193	103	1591
4	8	25	59	195	56	2320
5	4	n.a.*	35	n.a.*	14	n.a.*
6	2	6	10	48	2	238
7	4	8	41	42	27	342
8	5	35	15	466	70	3800
9	4	9	9	48	4	504
10	3	19	11	180	4	251
11	4	12	19	96	10	160
12	4	14	12	48	8	260
13	20	16	84	75	355	570
14	8	7	48	46	214	433
15	8	11	106	98	511	447

Table 3 The effect of BN administration on neutrophil luminol-amplified CL

N	Spont. 2nd visit	Spont. 3d visit	PMA-activ. 2nd visit	PMA-activ. 3d visit	Zym-activ. 2nd visit	Zym-activ. 3d visit
1	7	512	14	630	0	8760
2	5	2010	20	1766	23	7921
3	14	1407	418	1474	170	9345
4	12	1235	89	1081	220	12300
5	340	n.a.*	320	n.a.*	468	n.a.*
6	140	198	113	200	60	2540
7	84	501	150	785	2522	15054
8	85	2110	512	2280	25	28932
9	45	1094	216	2475	27	13709
10	134	932	465	1955	119	26411
11	122	618	491	1489	35	25585
12	320	608	51	1849	90	26144
13	771	796	2748	7615	10531	23739
14	416	797	8315	8731	19486	16523
15	675	38	5163	119	13340	214

Table 4 Effect of BN administration on neutrophil lucigenin-amplified CL

N	Spont. 2nd visit	Spont. 3d visit	PMA-activ. 2nd visit	PMA-activ. 3d visit	Zym-activ 2nd visit	Zym-activ. 3d visit
1	10	43	35	400	18	1900
2	8	205	22	418	24	2618
3	5	63	13	169	27	2587
4	2	71	18	171	48	3228
5	52	n.a.*	12	n.a.*	25	n.a.*
6	25	7	8	55	13	424
7	17	3	20	143	17	1673
8	15	35	26	466	140	3800
9	10	34	27	148	24	1847
10	22	50	78	486	7	2357
11	18	41	110	167	9	1966
12	30	43	72	151	32	2259
13	88	56	197	373	1702	2963
14	74	26	309	454	1536	1655
15	25	8	185	63	2240	74

Table 5 Effect of BN administration on the contents of SODs and catalase in the whole blood and leukocytes

N	SOD in the whole blood $\mu\text{g}/\text{mg}$ protein		Catalase in the blood $\mu\text{g}/\text{g}$ Hb		Leukocyte CuZnSOD ng/mg protein		Leukocyte MnSOD ng/mg protein	
	2nd visit	3d visit	2nd visit	3d visit	2nd visit	3d visit	2nd visit	3d visit
1	45	56	25	17	474	70	380	327
2	56	50	27	26	117	177	584	76
3	43	23	38	27	64	63	569	80
4	36	58	26	19	95	102	171	69
5	50	n.a.*	30	n.a.*	159	n.a.*	45	n.a.*
6	46	47	29	21	343	82	115	172
7	50	40	24	27	0	184	220	106
8	43	48	29	18	552	98	46	82
9	70	85	29	33	185	232	228	274
10	95	80	22	21	327	77	104	248
11	108	135	28	24	167	0	304	160
12	61	128	30	26	418	890	340	521
13	62	92	27	23	35	29	293	372
14	53	90	24	28	116	475	205	158
15	54	86	23	31	45	193	182	111

Table 6 Effect of BN administration on lymphocyte subpopulations (%)

N	CD3+		CD5+		CD4+		CD8+		CD14+		CD4+/CD8+	
	2nd visit	3d visit	2nd visit	3d visit	2nd visit	3d visit	2nd visit	3d visit	2nd visit	3d visit	2nd visit	3d visit
1	67.8	58.5	55.6	50.6	40.8	45.6	27.9	28.0	2.6	6.4	1.46	1.63
2	71.4	58.4	54.8	60.0	36.2	18.9	28.3	11.1	5.6	14.2	1.28	1.70
3	62.6	61.4	62.2	61.0	45.2	42.1	27.9	14.3	6.5	11.0	1.62	2.94
4	32.5	71.7	41.9	70.0	43.0	42.9	27.4	27.1	7.6	22.2	1.57	1.58
5	10.0	n.a.*	41.9	n.a.*	18.4	n.a.*	14.2	n.a.*	7.0	n.a.*	1.30	
6	40.5	62.9	52.8	54.5	33.4	42.6	17.0	18.6	7.7	12.3	1.96	2.29
7	67.5	67.8	45.6	50.4	42.6	34.7	28.6	33.2	7.3	10.2	1.50	1.05
8	74.0	66.4	63.5	62.4	42.6	15.3	29.0	16.1	10.9	12.0	1.47	0.95
9	22.9	72.5	21.9	66.4	20.4	41.6	22.1	18.9	4.1	5.8	0.92	2.20
10	61.3	53.5	63.3	46.2	31.5	28.2	29.2	40.8	12.2	4.7	1.08	0.69
11	60.5	52.0	55.5	49.0	32.2	40.1	18.5	33.7	1.3	10.8	1.74	1.19
12	56.8	57.0	62.7	60.0	26.0	30.0	35.7	34.0	11.5	9.0	0.73	0.88
13	71.3	80.6	54.0	73.0	49.9	58.8	21.9	18.6	4.5	3.7	2.28	3.16
14	76.3	68.3	70.3	66.0	48.9	48.0	25.3	22.1	9.6	4.3	1.93	2.17
15	52.6	60.2	54.9	56.7	26.8	41.9	15.5	12.0	7.7	4.3	1.73	3.49

Table 7 Effect of BN administration on lymphocyte and monocyte subpopulations (%)

N	3F3		NK		CD14+ lymphocytes		CD 14+ monocytes	
	2nd visit	3d visit	2nd visit	3d visit	2nd visit	3d visit	2nd visit	3d visit
1	19.0	11.0	25.8	19.5	2.6	6.4	72.4	47.4
2	17.3	10.6	18.3	15.5	5.6	14.2	72.3	56.0
3	11.4	8.4	23.4	20.8	6.5	11.0	65.4	61.2
4	18.7	26.0	25.6	21.8	7.6	22.2	46.2	63.5
5	34.4	n.a.	8.9	n.a.	7.0	n.a.*	45.9	n.a.
6	23.9	12.7	32.4	27.2	7.7	12.3	55.8	84.3
7	12.1	10.3	13.6	20.8	7.3	10.2	65.9	80.2
8	10.1	7.4	22.0	9.2	10.9	12.0	60.2	83.6
9	17.5	23.5	14.3	14.3	4.1	5.8	13.3	76.1
10	17.5	9.5	18.6	21.0	12.2	4.7	43.9	25.6
11	14.2	10.0	10.4	17.7	1.3	10.8	37.6	60.1
12	13.8	11.0	20.4	19.0	11.5	9.0	48.1	60.0
13	8.1	8.6	24.4	10.3	4.5	3.7	66.0	78.6
14	11.7	25.9	21.0	20.7	9.6	4.3	84.3	77.4
15	14.7	14.2	18.4	8.6	7.7	4.3	61.3	85.6

Table 8 Effect of BN administration on the contents of immunoglobulins

N	IgG (g/L)		IgA (g/L)		IgM (g/L)	
	2nd visit	3d visit	2nd visit	3d visit	2nd visit	3d visit
1	12.0	11.5	1.05	1.20	1.45	1.90
2	7.6	5.8	1.0	0.97	1.27	1.13
3	6.9	6.0	2.6	2.1	1.60	1.32
4	14.5	14.0	1.8	1.8	2.4	2.4
5	10.0	n.a.*	3.0	n.a.*	3.8	n.a.*
6	14.5	13.3	2.6	2.6	1.7	1.7
7	8.2	8.3	3.2	3.0	1.2	1.3
8	14.0	15.5	1.9	1.9	2.4	2.6
9	14.5	12.0	3.8	3.6	1.8	1.6
10	19.5	17.0	2.6	2.3	2.4	2.1
11	14.5	14.5	3.6	3.3	2.3	2.3
12	11.0	10.5	1.5	1.4	1.9	1.5
13	9.0	8.6	2.9	2.6	1.2	1.1
14	8.6	7.9	2.0	1.9	1.2	1.3
15	5.6	7.9	1.2	1.9	1.1	1.3

Table 9. Diabetes mellitus markers in the blood.

Number	Glucose content (mmol/L)		Glycosylated hemoglobin (%)		Keton content (mmol/L)	
	Before BN	After BN	Before BN	After BN	Before BN	After BN
1	8.4	6.2	9.2	8.4	0	0
2	6.6	10.4	11.6	9.2	0	0
3	5.3	5.1	10.8	8.1	0	0
4	5.6	4.6	10.2	9.1	0	0
5	14.5	n.a.	9.9	n.a.	9	n.a.
6	8.4	6.6	10.9	10.4	0	0
7	7.8	6.8	11.3	9.6	0	0
8	7.3	5.5	12.2	9.8	5.4	3.2
9	5.8	5.6	5.2	4.8	0	0
10	7.8	7.4	9.4	10.5	0	0
11	8.8	7.6	13.4	13.2	8	6
12	6.6	5.0	10.2	9.8	0	0
13	8.2	7.6	9.8	9.7	2.5	1.0
14	6.0	5.8	9.0	9.1	0	0
15	7.8	4.6	14.0	10.2	0	0

7.6 ± 2.2 6.3 ± 1.5 10.5 ± 2.0 9.4 ± 1.8

Table 10. Diabetes mellitus markers in urine.

Number	Glucose content (g/L)		Microalbumin (mg/L)		Urine output a day (L)	
	Before BN	After BN	Before BN	After BN	Before BN	After BN
1	0	0	180	160	2100	1600
2	23	8	340	300	1800	1500
3	30	19	0	0	2600	1900
4	15	0	0	0	2600	1900
5	88	n.a.	250	n.a.	4000	n.a.
6	28	15	200	156	2200	1850
7	39	17.5	150	120	2000	1600
8	36	6	95	60	2500	2200
9	0	0	0	0	1350	1250
10	36	24	350	360	2500	2200
11	70	50	n.a.	n.a.	2100	1500
12	29.5	22.2	0	0	2100	1800
13	31	21	150	130	2500	2200
14	20	22	0	0	1670	1700
15	42	12.5	0	0	2350	2000

325 ± 2.3 15.5 ± 1.3 122 ± 12.9 99 ± 11 2290 ± 590 1800 ± 292
 $p < 0.01$ $p > 0.05$ $p < 0.01$

Table 11. Clinical symptoms of diabetes mellitus.

Number	Insulin requirement (U/day)		Liver enlargement (cm below costal line)		Pancreas disorders	
	Before BN	After BN	Before BN	After BN	Before BN	After BN
1	0	0	0	0	-	-
2	44	28	2.0	1.5	+	-
3	28	28	2.5	0	-	-
4	42	36	0	0	+	-
5	32	n.a.	0	n.a.	-	n.a.
6	28	24	1.0	0	-	-
7	36	30	3.0	0	-	-
8	32	28	1.5	0	-	-
9	28	28	0	0	-	-
10	42	40	1.0	0	-	-
11	40	36	1.0	1.0	-	-
12	30	28	0	0	-	-
13	32	32	0	0	-	-
14	32	24	0	0	-	-
15	26	20	0	0	-	-

31 ± 10 27 ± 9

$p < 0.05$