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Bio-normalizer as a hypoallergic drug

Korkina LG, et. al

TRIAL PROTOCOL

(confidential)

Pilot Clinical Trial (Phase II)

Title: **BIO-NORMALIZER AS A HYPOALLERGIC DRUG**

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1.0 Checklist for Patient Eligibility and Necessary Information

Age between 2-15 years.
Wheezing
Attacks of breathlessness
"Normal" breathing before asthma attacks
Asthma attacks occuring more than one during previous year
Symptome-provoking factors (allargens, irritants, execise, etc.)
Bronchial asthma since less than 5 years
Informed consent explained to and signed by patient/parent.

Age between 2-15 years.
Atopic dermatitis
Skin lesions
Symptome-provoking factors (allargens, irritants, execise, etc.)
Symptomes of dermatitis since less than 5 years
Informed consent explained to and signed by patient/parent.

2.0 Objectives and Rationale

2.1 To determine the hypoallergic effects of Bio-normalizer (BN) on the patients suffered from atopic bronchial asthma.
2.2 To determine the hypoallergic effects of Bio-normalizer (BN) on the patients suffered from allergic dermatitis.
2.3 To study the effects of BN on the free radical status of children with atopic asthma and allergic dermatitis.

3.0 Background

Atopic asthma and allergic dermatitis are inflammatory diseases, which are initiated by the intake of an allergen or irritating agents. Although the mechanisms of developing these pathologies are not fully understood, there is a lot of up-to-date important information concerning major stimulating factors. Thus, it has been shown that an inflammatory reaction in the lungs may be mediated by the immunoglobulin E (IgE) [1] and depends on the amount of eosinophils in the peripheral blood [2]. However, it has now been proposed that in both cases free radicals play an important role [3]. Lulich et al. [4] assumed that the release of oxygen radicals is a primary source of β-adrenergic receptor dysfunction in asthmatic diseases. Indeed, Engels et al. [5] have earlier shown that the impairment of the β -adrenoceptor response depends on the release of oxygen radicals from stimulated macrophages. Furthermore, it has been shown [6] that IgE stimulates the superoxide release by monocytes from patients with asthma and rhinitis. Matsuyama et al. [7] showed an importance of oxygen radical generation in the rat model of allergic asthma and the induction of MnSOD during the disease development. Jarjour and Calhoun [8] have studied the production of superoxide by (~~airspace~~ alveolar) cells in 56 patients with asthma as compared with 49 normal controls. It was concluded that worsening of airway obstruction in asthma is associated with increased spontaneous generation of superoxide by (~~airspace~~ the lung) leukocytes.

Similarly, an essential role of oxygen radicals has been shown in skin inflammation. Thus, it was found that monocytes of patients with atopic dermatitis are primed for superoxide production [9] that can explain the overproduction of oxygen radicals during this pathology. Enhanced in vivo superoxide production from rat skin was shown in the case of UV-dependent fluoroquinolone-induced dermatitis [10]. Toxic effect of oxygen radicals in skin inflammation enhanced in the presence of iron [11].

Importance of free radical formation in inflammatory processes points out at the possibility of application of antioxidants and free radical scavengers for the treatment of atopic asthma and dermatitis. It is of special interest that N-acetylcysteine, a widely used mucolytic drug, is simultaneously an effective antioxidant. Among the other antiinflammatory drugs, salicylate, ibuprofen, ketoprofen, and indomethacin are also hydroxyl radical scavengers. Presently, various antioxidants and free radical scavengers are investigated as new antiinflammatory pharmacological agents. As it was pointed out, a special attention is drawn to N-acetylcysteine (NAC). Thus, Bernard [12] applied intravenous NAC to patients with established adult respiratory distress syndrome (ADRS) and showed that the NAC treatment permitted to increase significantly the diminished plasma and red cell glutathione levels in these patients. Leff et al. [13] showed that the postinsult treatment of rats with NAC decreased IL-1 induced leukocyte influx and lung leak. In the last studies recombinant SOD [14] and lipoic acid [15] were applied for the treatment lung injury and skin inflammation, respectively.

In accord with the above findings, in present clinical trial we have studied the hypoallergic effects of Bio-Normalizer, a natural food supplementation, on the patients with atopic asthma and allergic dermatitis. It has previously been shown that BN is a free radical scavenger [16,17] and a modulator of oxygen radical production by neutrophils and macrophages [18]. These findings suggest that BN may suppress oxygen radical-mediated inflammatory processes in the lung and skin.

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LIST OF PATIENTS

No	Name	Sex, Age (Years)	Diagnosis
1	Volkov S.	M (9)	Atopic bronchial asthma, the mild form, remission. Atopic dermatitis. Metabolic nephropathy
2	Osmanova O.	F (13)	Bronchial asthma, the mixed severe form, multi-relapsed. Post-hypoxic encephalopathy, rehabilitation . Spastic tetraparesis
3	Nemchikov D.	M (15)	Acute atopic dermatitis, bronchial asthma, the mixed form, multi-relapsed. Allergic rhinitis and sinusitis. Allergy to the grass pollen.
4	Semenov I.	M (6)	Bronchial asthma,the mixed severe form, unstable remission. Chronical gastritis.
5	Kashperov A.	M (10)	Brochial asthma, the mixed severe form, unstable remission. Cronical gastritis and cholecystitis.
6	Tarakonova I.	F (4)	Atopic dermatisis, acute form. Atopic bronchial asthma, the mixed severe form, frequent attacks of breathlessness. Allergic rhinitis
7	Shapochkina A.	F (9 m)	Child exema. Iron-deficient anemia.
8	Zhiganov A.	M (8)	Bronchial asthma, the mixed severe form
9.	Veselova A.	F (5)	Acute atopic dermatitis, unstable remission
10	Kumerin A.	M (13)	Mild bronchial asthma, the mixed form, remission.
11	Nikolko S.	M (15)	Atopic acute dermatitis, localized moderate form. Allergic rhinitis, sinusitis, and conjunctivitis.
12	Borschova T.	F (1 y, 2 m)	Infantile eczema, severe form, infection foci. Polyvalent allergy.
13	Atamanenko E.	F (13)	Atopic dermatitis, allergic rhinitis.
14	Kholmachev A.	M (1 y, 5 m)	Infantile eczema, severe form. Allergy to cow milk proteins. Perinatal encephalopathy. Left side hemiparesis.
15	Savel'ev A.	M (3 y, 5 m)	Local atopic dermatitis, acute moderate stage.
16	Lyzhin D.	M (7)	Atopic bronchial asthma, the mixed mild form.
17	Orlov N.	M (5)	Atopic bronchial asthma, the mixed form, remission
18	Barsukova T.	F (10)	Atopic bronchial asthma, the mixed moderate form.
19	Zyganov A.	M (13)	Atopic bronchial asthma, the mixed acute form.

20	Lazykin N.	M (3 y, 8 m)	Acute atopic dermatitis, moderate form. Bronchial asthma, the mixed moderate form, unstable remission.
21	Burdina T.	F (11)	Acute atopic dermatitis, moderate form. Bronchial asthma, the mixed severe form. Allergic rhinitis.
22	Razumovskii A.	M (4)	Atopic bronchial asthma, the mixed form, remission
23	Matkov A.	M (8)	Atopic bronchial asthma, the mixed severe form. Local atopic dermatitis, moderate form. Allergic rhinitis and sinusitis.
24	Gravanov S.	M (12)	Atopic dermatitis, remission. Atopic bronchial asthma, moderate form, remission.
25	Pankratov A.	M (8)	Atopic brochial asthma, the mixed severe form. Food allergy.
26	Koreshkov V.	M (4)	Atopic diffuse dermatitis, acute form. Atopic bronchial asthma, the mixed mild form, remission.
27	Andronova N.	F (5)	Local atopic dermatitis, acute form.
28	Shashkin A.	M (12)	Atopic diffuse dermatitis, acute moderate form.
29	Perfil'ev A.	M (8)	Atopic bronchial asthma, the mixed moderate form, unstable remission. Allergy to grass pollen. Allergic rhinitis.
30	Legkun G.	M (4)	Atopic bronchial asthma, the mixed moderate form, remission

Children with the odd numbers received BN, while those with the even numbers do not receive it.

TRIAL DESIGN

Group 1
Patients with bronchial asthma received BN
N1 Volkov
N5 Kashperov
N17 Orlov
N19 Zyganov
N23 Matkov
N25 Pankratov
N29 Perfil'ev

Group 2
Patients with atopic dermatitis received BN
N3 Nemchikov
N7 Shaposhnikiva
N9 Veselova
N11 Nikolko
N13 Atamanenko
N15 Savel'ev
N21 Burdina
N27 Andronova

Group 3
Patients with bronchial asthma, who did not receive BN (Control)
N2 Osmanova
N4 Semenov
N8 Zhiganov
N10 Kumerin
N16 Lyzhin
N18 Barsukova
N22 Razumovskii
N30 Legkun

Group 4
Patients with atopic dermatitis, who did not receive BN (Control)
N6 Tarakanova

N12 Borschova
N14 Cholmachev
N20 Lazykin
N24 Gravanov
N26 Koreshkov
N28 Shashkin

Frequency of bronchial asthma attack (breathlessness)

Table 1 The first visit

Frequency of attack	0	1-2	3-5	>5
Group 1 (asthma + BN)	3/7 (43%)	1/7 (14%)	0	3/7 (43%)
Group 3 (asthma - BN)	4/8 (50%)	2/8 (25%)	0	2/8 (25%)
Group 2 (dermat. + BN)				
Group 4 (dermat. - BN)				

Table 2 The second visit

Frequency of attack	0	1-2	3-5	>5
Group 1 (asthma + BN)	5/7 (71%)	1/7 (14%)	1/7 (14%)	0
Group 3 (asthma - BN)	4/8 (50%)	3/8 (38%)	0	1/8 (13%)
Group 2 (dermat. + BN)				
Group 4 (dermat. - BN)				

Table 3 The third visit

Frequency of attack	0	1-2	3-5	>5
Group 1 (asthma + BN)	6/7 (86%)	1/7 (14%)	0	0
Group 3 (asthma - BN)	6/8 (75%)	1/8 (13%)	0	1/8 (13%)
Group 2 (dermat. + BN)				
Group 4 (dermat. - BN)				

Dynamics of wheezing

Table 4

	1th visit	2nd visit	3d visit
Group 1 (asthma + BN)	5/7 (71%)	2/7 (29%)	0
Group 3 (asthma - BN)	4/8 (50%)	2/8 (25%)	1/8 (13%)

Number of necessary weekly β_2 agonist puffs

Table 5 The first visit

Number of inhalations	0	<3	3-7	>7
Group 1 (Asthma + BN)	2/7 (29%)	0	1/7 (14%)	4/7 (57%)
Group 3 (Asthma - BN)	4/8 (50%)	0	0	4/8 (50%)

Table 6 The second visit

Number of inhalations	0	<3	3-7	>7
Group 1 (Asthma + BN)	5/7 (71%)	0	0	2/7 (29%)
Group 3 (Asthma - BN)	4/8 (50%)	0	0	4/8 (50%)

Table 7 The third visit

Number of inhalations	0	<3	3-7	>7
Group 1 (Asthma + BN)	6/7 (86%)	0	0	1/7 (14%)
Group 3 (Asthma - BN)	3/8 (38%)	0	1/8 (12%)	4/8 (50%)

FEV_1 Dynamics

Table 8 Group 1 (Asthma + BN)

	The first visit		The second visit		The third visit	
No of patient	FEV_1 value	% of expected value	FEV_1 value	% of expected value	FEV_1 value	% of expected value
1	2.178	91.0	1.969	81.9	2.090	87.0
5	2.299	82.0	2.486	89.0	2.420	86.6
17	1.450	104.3	1.360	97.8	1.380	99.3
19	1.232	36.7	1.485	44.2	1.562	46.5
23	1.492	46.4	1.511	58.7	1.524	64.5
25	1.144	55.6	1.804	87.7	1.904	92.5
29	1.859	90.4	1.861	92.3	1.863	93.7
	m ± SD	72.3 ± 25.8			m ± SD	81.4 ± 18.9

Table 9 Group 3 (asthma - BN)

	The first visit		The second visit		The third visit	
No of patient	FEV_1 value	% of expected value	FEV_1 value	% of expeted value	FEV_1 value	% of expected value
2	1.265	51.3	1.930	37.7	1.518	61.6
4	1.474	86.2	1.089	73.9	1.510	88.3
8	0.572	29.0	1.800	75.1	1.820	77.3
10	2.352	93.7	2.358	94.3	2.362	95.2
16	1.914	83.3	1.923	85.6	1.928	87.3
18	1.389	69.0	1.389	69.0	1.455	75.6
	m ± SD	68.8 ± 24.7			m ± SD	80.9 ± 11.9

In the case of N22 and N30 the determination of FEV1 was impossible due to a small age.

Table 10 The eosinophil content in the blood of children of Group 1 (asthma + BN)

No of patient	The first visit	The third visit
1	8	6
5	11	10
17	13	9
19	13	11
23	13	14
25	12	7
29	12	9

Table 11 The eosinophil content in the blood of children of Group 3 (asthma - BN)

No of patient	The first visit	The third visit
2	5	7
4	10	4
8	9	7
10	4	3
16	9	8
18	10	6
22	6	5
30	6	5

Table12 The eosinophil content in the blood of children of Group 2 (dermatitis + BN)

No of patient	The first visit	The third visit
3	10	8
7	26	23
9	5	2
11	11	8
13	5	2
15	15	17
21	13	4
27	12	10

Table 13 The eosinophil content in the blood of children of Group 4 (dermatitis - BN)

No of patient	The first visit	The third visit
6	11	5
12	31	8
14	13	12
20	5	6
24	3	4
26	9	12
28	7	5

Table 14 The IgE content in the serum of children of Group 1 (asthma + BN)

No of patient	The first visit	The third visit
1	356	263
5	1547	914
17	319	286
19	322	356
23	301	290
25	325	249
29	243	199

Table 15 The IgE content in the serum of children of Group 3 (asthma - BN)

No of patient	The first visit	The third visit
2	36	22
4	361	380
8	100	101
10	128	200
16	235	198
18	328	255
22	245	206
30	189	176

Table 16 The IgE content in the serum of children of Group 2 (dermatitis + BN)

No of patient	The first visit	The third visit
3	292	261
7	2405	275
9	67	73
11	188	203
13	64	61
15	300	202
21	380	246
27	263	187

Table 17 The IgE content in the serum of children of Group 4 (dermatitis - BN)

No of patient	The first visit	The third visit
6	182	178
12	289	238
14	100	105
20	318	312
24	309	298
26	108	124
28	275	246

Table 18 **Luminol-amplified CL Group 1 (asthma + BN)**

N of patient	Spontaneous 1th day	Spontaneous 30th day	Stimulated 1th day	Stimulated 30th day
1	63	20	1320	843
5	400	19	2220	120
17	26	25	266	774
19	12	54	127	2290
23	204	32	1860	390
25	27	17	238	199
29	84	13	972	218

Table 19 **Lucigenin-amplified CL Group 1 (asthma + BN)**

N of patient	Spontaneous 1th day	Spontaneous 30th day	Stimulated 1th day	Stimulated 30th day
1	2	0	81	60
5	7	3	148	28
17	5	2	20	43
19	1	3	11	120
23	4	3	86	30
25	2	3	25	27
29	2	2	105	28

Table 20 **Luminol-amplified CL Group 3 (asthma - BN)**

N of patient	Spontaneous 1th day	Spontaneous 30th day	Stimulated 1th day	Stimulated 30th day
2	13	28	625	502
4	5	61	182	614
8	77	42	483	445
10	113	15	2040	1554
16	42	65	214	312
18	7	17	264	340
22	7	9	226	630
)	23	9	4554	753

Table 21 **Lucigenin-amplified CL Group 3 (asthma - BN)**

N of patient	Spontaneous 1th day	Spontaneous 30th day	Stimulated 1th day	Stimulated 30th day
2	2	2	54	40
4	1	3	614	64
8	5	4	36	51
10	4	5	91	102
16	4	6	32	42
18	1	2	29	26
22	1	2	20	65
30	3	4	310	115

Table 22 **Luminol-amplified CL Group 2 (dermatitis + BN)**

N of patient	Spontaneous 1th day	Spontaneous 30th day	Stimulated 1th day	Stimulated 30th day
3	80	80	203	220
7	58	3	494	39
9	4	27	224	1175
11	21	52	1256	1369
13	19	30	108	273
15	63	78	270	340
21	75	66	297	1440
27	10	24	118	260

Table 23 **Lucigenin-amplified CL Group 2 (dermatitis + BN)**

N of patient	Spontaneous 1th day	Spontaneous 30th day	Stimulated 1th day	Stimulated 30th day
3	2	3	22	29
7	4	2	52	23
9	1	2	18	84
11	2	3	58	119
13	4	5	19	42
15	2	2	31	40
21	3	6	28	146
27	2	3	15	25

Table 24 **Luminol-amplified CL Group 4 (dermatitis - BN)**

N of patient	Spontaneous 1th day	Spontaneous 30th day	Stimulated 1th day	Stimulated 30th day
6	21	34	268	417
12	73	9	910	186
14	4	6	44	69
20	22	57	138	1219
24	30	51	1290	1230
26	85	29	2550	1209
28	120	50	2608	800

Table 25 **Lucigenin-amplified CL Group 4 (dermatitis - BN)**

N of patient	Spontaneous 1th day	Spontaneous 30th day	Stimulated 1th day	Stimulated 30th day
6	2	6	16	53
12	9	1	154	15
14	2	3	10	17
20	2	7	16	98
24	5	8	121	152
26	5	8	118	132
28	3	4	230	131

Table 26 **The GSH and GSSG content (μmol/g Hb) Group 1 (asthma + BN)**

No of patient	GSH (1th day)	GSH (30th day)	GSSG (30th day)	GSSG/GSH (30th day)
1	8.8	8.1	0.0065	0.00080
5	9.6	9.1	0.0137	0.00150
17	5.9	8.3	0.0073	0.00088
19	7.6	6.6	-	-
23	7.7	7.1	0.0040	0.00056
25	7.9	9.3	0.0097	0.00104
29	7.7	7.7	0.0092	0.00120
	7.9±1.1	8.0±1.0	0.0084±0.0033	0.00100±0.0003

Table 27 **The GSH and GSSG content (μmol/g Hb) Group 3 (asthma - BN)**

No of patient	GSH (1th day)	GSH (30th day)	GSSG (30th day	GSSG/GSH (30th day)
2	8.1	8.6	0.0127	0.00148
4	7.2	7.5	0.0120	0.00160
8	7.9	8.1	0.0133	0.00164
10	7.8	6.5	0.0127	0.00195
16	6.4	6.4	0.0111	0.00174
18	7.5	8.2	0.0126	0.00154
22	7.5	7.5	0.0137	0.00180
30	8.6	8.1	0.0130	0.00160
	7,6 ± 0,6	7,6 ± 0,8	0,0126 ± 0,0007	0,00167 ± 0,00015

Table 28 The GSH and GSSG content (μmol/g Hb) Group 2 (dermatitis + BN)

No of patient	GSH (1th day)	GSH (30th day)	GSSG (30th day)	GSSG/GSH (30th day)
3	5.3	5.3	0.0090	0.00170
7	10.5	6.6	0.0087	0.00132
9	8.3	6.1	0.0133	0.00220
11	7.9	7.2	0.0100	0.00139
13	6.5	8.4	0.0163	0.00194
15	8.7	8.7	0.0077	0.00088
21	6.6	6.8	0.0110	0.00162
27	8.3	6.7	0.0087	0.00130
	7.8±1.6	7.0±1.1	0.0106±0.0029	0.00154±0.0004

Table 29 The GSH and GSSG content (μmol/g Hb) Group 4 (dermatitis - BN)

No of patient	GSH (1th day)	GSH (30th day)	GSSG (30th day)	GSSG/GSH (30th day)
6	6.8	8.6	0.0133	0.00154
12	8.1	7.1	0.0149	0.00210
14	8.7	8.4	0.0191	0.00227
20	5.4	7.7	0.0178	0.00230
24	9.4	7.7	0.0200	0.00260
26	6.3	8.1	0.0205	0.00250
28	7.0	7.0	0.0137	0.00200
	7.4±1.4	7.8±0.6	0.0170±0.003	0.00219±0.0004

Table 30 Dynamics of skin pruritus

	1th visit	2nd visit	3d visit
Group 2 (dermatitis+ BN)	7/8 (88%)	6/8 (75%)	2/8 (25%)
Group 4 (dermatitis - BN)	4/7 (57%)	3/7 (43%)	3/7 (43%)

Table 31 Dynamics of excoriation

	1th visit	2nd visit	3d visit
Group 2 (dermatitis+ BN)	8/8 (100%)	7/8 (88%)	3/8 (38%)
Group 4 (dermatitis - BN)	5/7 (71%)	5/7 (71%)	3/7 (38%)

Table 32 Score of a skin lesion square*

	1th visit	2nd visit	3d visit
Group 2 (dermatitis+ BN)	22/40 (55%)	18/40 (45%)	12/40 (30%)
Group 4 (dermatitis - BN)	12/35 (34%)	8/35 (23%)	8/35 (23%)

* It was assumed that score 1 corresponds to one lesion including a facial lesion, a neck lesion, a hand lesion, a foot lesion, and a trunk lesion. Thus, a total score for one patient is 5; so a total possible score for Group 2 (8 patients) is 40 and that for Group 4 (7 patients) is 35.

Table 33 The requirement in steroid ointment applications *)

	1th visit	2nd visit	3d visit
Group 2 (dermatitis+ BN)	10/16 (63%)	8/16 (50%)	1/16 (6%)
Group 4 (dermatitis - BN)	4/14 (28%)	4/14 (28%)	4/14 (28%)

*) It was assumed that score 0, 1, and 2 correspond to the application of steroid ointments for 0, <3, and >3 times a week, respectively.

Table 34 Antihistamine drug requirement

	1th visit	2nd visit	3d visit
Group 2 (dermatitis+ BN)	2/8 (25%)	4/8 (50%)	2/8 (25%)
Group 4 (dermatitis - BN)	3/7 (43%)	4/7 (57%)	3/7 (43%)

Table 35 The content of methemoglobin (MetHb) and plasma antioxidant activity (AOA) Group 1 (asthma + BN)

No of patient	MetHb (%) (1th day)	MetHb (%) (30th day)	AOA (1th day)	AOA (30th day)
1	30.0			
5	2.4	8.9	36.5	20.0
17	5.0	10.3	21.8	31.3
19	27.2	23.5	32.3	12.3
23	19.5	21.4	-1.9	1.7
25	21.9	23.4	25.4	25.3
29	21.0	12.9	22.3	12.7

Table 36 **The content of methemoglobin (MetHb) and plasma antioxidant activity (AOA) Group 3 (asthma - BN)**

No of patient	MetHb (%) (1th day)	MetHb (%) (30th day)	AOA (1th day)	AOA (30th day)
2	14.2	9.8	18.3	14.2
4	21.3	21.3	14.5	10.5
8	23.5	23.9	40.1	42.8
10	36.8	8.5	10.6	11.5
16	8.6		16.5	
18	18.7		19.0	
22	9.4	0.0	38.8	36.8
30	13.9	12.2	26.6	20.6

Table 37 **The content of methemoglobin (MetHb) and plasma antioxidant activity (AOA) Group 2 (dermatitis + BN)**

No of patient	MetHb (%) (1th day)	MetHb (%) (30th day)	AOA (1th day)	AOA (30th day)
3	12.8		13.3	
7	35.4	10.4	22.0	29.8
9	10.7	18.6	13.5	14.3
11	18.1	21.5	13.3	17.5
13	8.8	10.9	28.4	19.6
15	13.3		39.8	
21	3.3	9.0	45.3	9.1
27	10.9	20.6	21.4	24.5

Table 38 **The content of methemoglobin (MetHb) and plasma antioxidant activity (AOA) Group 4 (dermatitis - BN)**

No of patient	MetHb (%) (1th day)	MetHb (%) (30th day)	AOA (1th day)	AOA (30th day)
6	22.4	10.4	52.7	48.8
12	28.6	25.0	40.1	46.6
14	27.6	19.5		
20	6.6	12.3	2.8	28.1
24	20.9	14.0	25.2	26.3
26	12.4	11.8	22.0	14.6
28	19.2	12.8	16.7	15.9

CONCLUSIONS

1. One month Bio-Normalizer administration decreased significantly the frequency of bronchial asthma attacks and the b2-agonist requirement of children who suffered from atopic bronchial asthma.
2. The course of BN therapy suppressed skin lesions and other symptomes of atopic dermatitis and decreased the requirement in steroid oitment applications.
3. There were two drop-outs from the dermatitis group due to worsened clinical symptomes in children with severe food allergy after 3-5 days of the trial.
4. BN administration significantly improved children's antioxidant systems (superoxide dismutase and catalase activities, and glutathione metabolism).