IMPROVEMENT OF IMMUNE FUNCTION BY EM•X GOLD, A HEALTH DRINK CONTAINING EXTRACT FROM CULTURE OF EFFECTIVE MICROORGANISMS (ECEM)

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Abstract

Objectives: The objective of this study was to investigate the efficacy of EM•X GOLD, which is a health drink contains the extract from culture of effective microorganisms (ECEM), on human immunity.

Methods: A randomized, placebo-controlled, double blind intervention trial was conducted in 47 healthy subjects. They were randomly assigned to two groups and ingested either test drink containing ECEM or placebo for 12 weeks. Immunological vigor and anti-inflammation effect were set as the primary outcomes. Scoring of immunological vigor (SIV), a scoring system that can combine seven major immunological parameters, and the self-examination of immunological vigor (SEIV), were used to evaluate them. SIV also shows T lymphocyte age, which indicates the age evaluated from immunological vigor point of view.

Results: Of 47 applicants, 5 were excluded for various reasons, and 4 dropped out. Consequently 38 subjects completed the trial.

Among SIV, T lymphocyte age and $CD8^+CD28^+$ T cell showed significant improvement after the ingestion of EM•X GOLD 12 weeks when compared to the placebo.

No significant improvement was shown in SEIV.

Conclusions: These results suggest that the daily supplementation of EM•X GOLD can be effective to keep immunity function at a high level.

INTRODUCTION

Immune function is an important function for the human body to defend it from disease therefore it is highly desirable to keep it at a high level to lead healthy everyday lives. However, human faces the decline of the immune function since its peak at the age of 20, which is caused by simply advancing age, and by unavoidable matters in daily lives such as inadequate lifestyle or stress. That decline causes not only an increased risk for disease or infection but also less-resistivity against stress, and eventually it results in the deterioration of QOL (Quality of Life)[1)2)]. Therefore it should be reasonable to say that the proactive use of the food with immunostimulatory activity is effective for human body to keep immunity function at a high level.

The extract from culture of effective microorganisms (ECEM) is secondary metabolite produced in symbiotic culture of a mixture of 5 microorganisms; 2 species of yeasts (*Saccharomyces cerevisiae* and *Candida ethanolica*), 2 species of lactic acid bacteria (*Lactobacillus casei* and *Lactobacillus farraginis*) and photosynthetic bacteria (*Rhodopseudomonas palustris*). A health drink containing ECEM and named EM•X GOLD (EMXG) has been produced and brought to the market.

The recent research shows that the ECEM has a function of activating immune cells (macrophage)[3)]. It suggests ECEM can contribute to the immunity function of human body.

In this study, 12-week randomized, placebo-controlled, double-blind trial was conducted to investigate the effect on the immunological status of human ingestion of the drink containing ECEM by measuring the scoring of immunological vigor (SIV) score and other immunological, physical, biochemical, and subjective symptom parameters see an explanation of SIV below, 3. Outcomes).

Table 1 Composition of Investigational products

Components	EMXG	Placebo
Energy (kcal)	0	0
Protein (g)	0	0
Lipid (g)	0	0
Carbohydrates (g)	0	0
Na (mg)	6.0	3.0
ECEM (ml)	1.07	0

per 30 ml

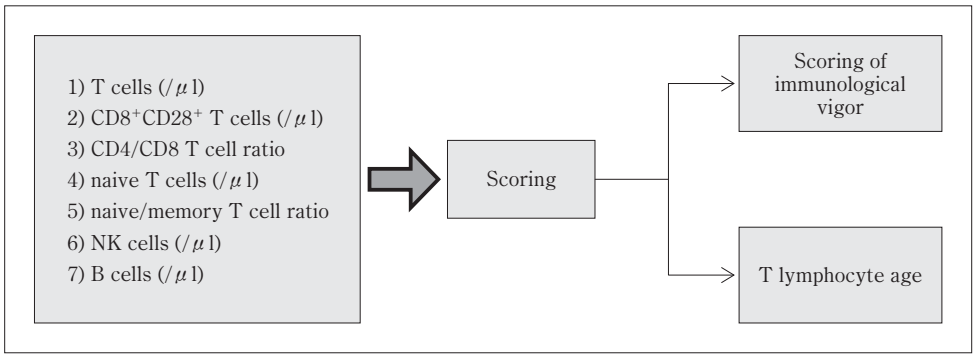

Fig. 1

Table 2　Schedule for the study

Item \ Term	Pre Trial Test	Screening	Test period (12 w)
			After 12 w
Informed consent	○		○
SIV	○		○
SEIV	○		○
Selection and/or allocation		○	
BAP and d-Roms	○		○
Biochemical analysis of the blood	○		○
Urine analysis	○		○
Ingestion of test drinks			◆——▶
Log			◆——▶

○ : Implementation

◆——▶ : Daily practice during the test period

MATERIALS AND METHODS

1. Investigational products

We prepared a drink, EM•X GOLD containing ECEM, manufactured by EM Research Organization Inc. for carrying out this study. A placebo drink was prepared without ECEM. Both drink looked identical. The nutritional constituent is shown **Table 1**.

2. Study design

This was a 12-week randomized, placebo-controlled, double-blind study examining the efficacy of EMXG ingestion on human immunity. This study was performed from January to April 2015. All of the business side was entrusted to Japan Clinical Trial Association (JACTA).

This study was conducted in accordance with the ethical principles of the declaration of Helsinki. The study protocol was approved by the Institutional Review Board of LLP Pharmaceutical Law Wisdoms (Tokyo), and written informed consent was obtained from all subjects.

Candidate subjects were male and female volunteers who were recruited by advertisement.

They were selected according to the following criteria.
1) Healthy Japanese men and women aged 30 to 59 years with daily tiredness and fatigue ;
2) With a relatively low Self-examination of immunological vigor (SEIV)[4] ≥90 (male), ≥100 (female); The exclusion criteria is as follows:
(1) With a relatively high SIV ≥ 19 ;
(2) Previously suffered malignant tumors, heart failure, or cardiac infarction;
(3) Under the care of a doctor for the treatment of chronic diseases such as atrial fibrillation, uneven heartbeat, rheumatism, diabetes, high blood pressure, and diseases of the liver, kidney, cerebral system, circulatory system, and lipid metabolism;
(4) Taking medicines, including herbal medicines;
(5) With pollen allergy;
(6) Pregnant, nursing, or were likely to become pregnant during the trial;
(7) Judged to be unsuitable to participate in the test by the doctor responsible for the present study;

The subjects were instructed as follows: to take the assigned items as indicated; to maintain their usual lifestyles and habits, avoiding too much food, drink, or alcohol; to avoid excessive exercise; and to keep a daily record that included the intake of the assigned item (or not) and lifestyle factors during the test period, and to send the diary by mail to the study coordinator every 7

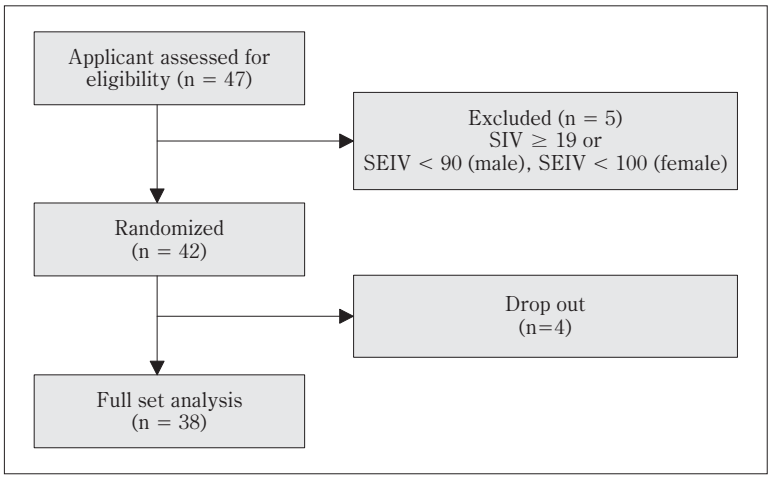

Fig. 2

Table 3 Background of the whole subject populations

Item	Unit	EMXG	Placebo
Number of subjects (gender)	psn.	21 (F 13, M 8)	17 (F 10, M 7)
Age	year	45.5 ± 5.1	43.5 ± 5.3
SIV	score	15.8 ± 1.6	16.2 ± 1.6
SEIV	score	111.3 ± 8.8	108.1 ± 8.2

Mean ± SD

No significant difference

days.

3. Outcomes

Immunological vigor and anti-inflammation effect were set as the primary outcomes. SIV and SEIV were used to evaluate them. SIV is the scoring system that can combine seven immunological parameters, the quantification of T cells, naive T cells, $CD8^+CD28^+$ T cells, B cells, and NK cells, the CD4/CD8 T cell ratio, and the naive/memory T cell ratio[5)6)], and express the immune status of individuals as a simple numeral. This system also shows T lymphocyte age. Due to this, we can compare the real age with the age evaluated from immunological vigor's point of view (**Fig.1**).

The SIV scoring system is an index system, which utilizes the database of test results for every particular item, which are accumulated by Hirokawa and Utsuyama, and by using the database its index is calculated from several subsets. Therefore it enables to evaluate the condition of a variety of immune cells in a comprehensive and multilateral manner, and it has been already introduced in several research as the new testing method of immune strength.

SEIV is a self-examination of immunological vigor with Likert scales.

Antioxidant and safety were set as the secondary outcomes.

The BAP and d-Roms of the blood were measured to evaluate antioxidant.

The biochemical analysis of the blood, the urine analysis and inquiry were done to assess safety.

According to the schedule shown in **Table 2**, we measured parameters on safety and efficacy.

4. Statistics

Data were expressed as Mean ± SD. For SIV, other immunological parameters and antioxidant, the changes from the baseline in the same group were assessed using paired t-test. And in the intergroup comparison, after confirmation if the changes from the baseline after the ingestion for 12 weeks were within normal distribution and equal variances, Student's t-test, Welch's t-test or Mann-Whitney U test were conducted. For SEIV, the changes from the baseline in the same group were assessed using Wilcoxon signed-rank test, and in the intergroup comparison, Mann-Whitney U test was used instead. For biochemical blood and urine analyses, the changes from the baseline in the same group were assessed using paired t-test (if $N < 10$, Wilcoxon signed-rank test was used instead.), and in the intergroup comparison, the Student's t-test was conducted. For assessment of the subjects background in the intergroup comparison, Student's t-test was conducted. The statistical analyses were performed with Statcel 3 (Yanai, 2011). $p < 0.05$ was considered significant.

Table 4　All subject

Item	Unit	Group	0 w	12 w	Between-group difference (P-value)
SIV	−	EMXG	15.8 ± 1.6	15.3 ± 1.4	0.311
		Placebo	16.2 ± 1.6	15.1 ± 2.0*	
T lymphocyte age	year	EMXG	53.6 ± 5.8	52.8 ± 6.8	0.019#
		Placebo	48.1 ± 8.5	51.2 ± 6.8*	
T cell	/μl	EMXG	1307.3 ± 325.4	1355.8 ± 369.9	0.235
		Placebo	1412.5 ± 392.0	1307.6 ± 322.4	
$CD8^+CD28^+$ T cell	/μl	EMXG	175.4 ± 59.4	191.6 ± 90.5	0.008##
		Placebo	237.1 ± 102.1	193.4 ± 70.5*	
CD4/CD8 T cell ratio	ratio	EMXG	3.30 ± 1.35	3.38 ± 1.32	0.649
		Placebo	3.14 ± 1.96	3.36 ± 2.29	
Naive T cell	/μl	EMXG	329.5 ± 171.0	308.3 ± 149.9	0.182
		Placebo	346.3 ± 139.1	271.8 ± 113.3*	
Naive/memory T cell ratio	ratio	EMXG	0.71 ± 0.53	0.62 ± 0.42	0.793
		Placebo	0.61 ± 0.21	0.50 ± 0.21**	
NK cell	/μl	EMXG	273.1 ± 207.5	203.7 ± 248.0*	0.749
		Placebo	219.8 ± 131.2	162.1 ± 116.8*	
B cell	/μl	EMXG	240.0 ± 118.4	263.2 ± 124.7	0.601
		Placebo	280.1 ± 129.2	289.3 ± 179.0	

EMXG n = 21, Placebo n = 17, Mean ± SD

* $p < 0.05$, ** $p < 0.01$ against baseline (0 w)

# $p < 0.05$, ## $p < 0.01$ between-group differences from baseline (0 w)

RESULTS

1. Panel demographics

Of 47 applicants for this study, 5 were excluded for various reasons. Overall, 42 applicants were randomly assigned to the interventions, and 4 dropped out of this study mainly due to personal reasons.

Consequently, 38 subjects, of whom 21 received EMXG and 17 received the placebo, completed the trial (**Fig.2**). There were no significant differences between the groups (**Table 3**).

2. SIV

The results of the statistical analysis of the SIV, T lymphocyte age and other immunological parameters are shown in **Table 4**.

As for SIV, the scores decreased significantly in the placebo group when compared with baseline data. In the EMXG group the scores did not decrease significantly.

As for T lymphocyte age, the aging was shown significantly in the placebo group when compared with baseline data, meanwhile in the EMXG group the aging improved. There were the significant differences between two groups for 12 weeks ingestion ($p = 0.019$).

For the immunological parameters, $CD8^+CD28^+$ T cell decreased significantly in the placebo group when compared with baseline data, meanwhile in the EMXG group increased over the ingestion period. $CD8^+CD28^+$ T cell showed significant improvement after the ingestion of EMXG for 12 weeks when compared to the placebo group ($p = 0.008$).

Also, the within-group analysis showed that only in the placebo group Naive T cell and Naive/memory T cell ratio decreased significantly after supplementation ($p < 0.05$).

3. SEIV and Antioxidant

For SEIV and antioxidant, there could not be found the significant differences after the ingestion of EMXG for 12 weeks when compared to the placebo group (Data not shown).

4. Safety assessments

No severe changes were detected on the biochemical analysis of the blood (**Table 5**) and the urine analysis (**Table 6**). Some variables changed, significantly from baseline after test food ingestion, but only slightly and within the normal range or were judged as temporary.

No adverse effects attributable to EMXG ingestion were found.

DISCUSSION

The purpose of this study is to examine the effect on the immunological status of human ingestion of the drink containing ECEM.

In this study, the comprehensive immunological status of each individual was measured with the SIV method.

Table 5 Transition of Biochemical Blood Test

Item	Unit	Std. Value	Gender	Group	0 w	12 w
Total Bilirubin	mg/dL	0.2–1.2	M/F	EMXG Placebo	0.69 ± 0.27 0.62 ± 0.25	0.62 ± 0.29 0.68 ± 0.29
Total Protein	g/dL	6.5–8.3	M/F	EMXG Placebo	7.3 ± 0.4 7.5 ± 0.4	7.1 ± 0.4 7.0 ± 0.4** #
Albumen	g/dL	3.8–5.3	M/F	EMXG Placebo	4.7 ± 0.3 4.6 ± 0.4	4.7 ± 0.3 4.5 ± 0.4
AST (GOT)	U/L	8–38	M/F	EMXG Placebo	21.7 ± 5.9 19.2 ± 3.7	21.1 ± 5.3 19.1 ± 3.8
ALT (GPT)	U/L	4–43	M/F	EMXG Placebo	19.2 ± 12.0 18.2 ± 5.6	19.1 ± 9.7 17.6 ± 6.7
ALP	U/L	110–354	M/F	EMXG Placebo	190.8 ± 56.2 201.5 ± 45.5	196.2 ± 55.7 203.6 ± 52.6
LD (LDH)	U/L	121–245	M/F	EMXG Placebo	189.1 ± 29.5 177.4 ± 26.7	176.9 ± 23.6** 167.4 ± 23.8*
γ -GT (γ GTP)	U/L	86 and under	M	EMXG Placebo	62.3 ± 56.1 28.7 ± 24.1	55.4 ± 51.9 32.1 ± 38.9
		48 and under	F	EMXG Placebo	22.0 ± 8.5 20.3 ± 7.8	22.6 ± 9.0 17.1 ± 5.9**
CK (CPK)	U/L	38–196	M	EMXG Placebo	188.5 ± 76.9 151.7 ± 120.5	173.0 ± 67.8 105.1 ± 44.6
		30–172	F	EMXG Placebo	107.7 ± 50.1 93.2 ± 38.0	97.6 ± 40.7 135.8 ± 155.3
Total Cholesterol	mg/dL	130–219	M/F	EMXG Placebo	216.8 ± 36.6 202.6 ± 44.8	212.9 ± 22.3 199.8 ± 40.8
Neutral Fat (TG)	mg/dL	30–149	M/F	EMXG Placebo	87.8 ± 42.5 104.0 ± 67.9	97.8 ± 59.2 113.8 ± 54.5
Sodium	mEq/L	135–150	M/F	EMXG Placebo	142.5 ± 1.9 141.5 ± 2.2	145.6 ± 1.5** 145.4 ± 2.2**
Chloride	mEq/L	98–110	M/F	EMXG Placebo	103.6 ± 2.8 103.1 ± 1.9	106.1 ± 1.5** 106.7 ± 1.8**
Potassium	mEq/L	3.5–5.3	M/F	EMXG Placebo	4.8 ± 0.9 5.0 ± 1.0	4.2 ± 0.4** 4.3 ± 0.3**
Calcium	mg/dL	8.4–10.2	M/F	EMXG Placebo	9.4 ± 0.4 9.5 ± 0.4	9.5 ± 0.3 9.4 ± 0.5
Inorganic Phosphorus	mg/dL	2.5–4.5	M/F	EMXG Placebo	3.5 ± 0.3 3.6 ± 0.4	3.7 ± 0.5 3.6 ± 0.5
Urea Nitrogen	mg/dL	8.0–22.0	M/F	EMXG Placebo	14.2 ± 3.4 13.8 ± 2.5	14.3 ± 3.8 13.0 ± 3.0
Creatinine	mg/dL	0.61–1.04	M	EMXG Placebo	0.83 ± 0.15 0.77 ± 0.15	0.85 ± 0.16 0.77 ± 0.09
		0.47–0.79	F	EMXG Placebo	0.59 ± 0.06 0.63 ± 0.09	0.62 ± 0.07 0.60 ± 0.08* #
Blood Sugar (Serum)	mg/dL	60–109	M/F	EMXG Placebo	55.8 ± 12.0 60.7 ± 10.9	75.2 ± 17.0** 72.9 ± 8.3**

EMXG n = 21, Placebo n = 17, Mean ± SD

* $p < 0.05$, ** $p < 0.01$ against baseline (0 w)

# $p < 0.05$ between-group differences from baseline (0 w)

Table 6　Transition of Urinalysis

Item	Unit	Std. Value	Gender	Group	0 w	12 w
Specific Gravity	mg/dL	1.010–1.025	M/F	EMXG Placebo	1.0181 ± 0.0060 1.0212 ± 0.0072	1.0195 ± 0.0059 1.0188 ± 0.0063
pH	g/dL	4.5–8.0	M/F	EMXG Placebo	6.3 ± 0.7 6.0 ± 0.6	5.7 ± 0.6* 5.7 ± 0.5*

EMXG n = 21, Placebo n = 17, Mean ± SD
* $p < 0.05$ against baseline (0 w)
between-group differences from baseline (0 w)

SIV score is comprehensive index scores of items easily reduced by aging or stress out of various functions of immune function, therefore that reflects the state of aging or stress[7].

In this study, a placebo-controlled double-blind trial revealed that the ingestion of EMXG for 12 weeks significantly improved the SIV score, particularly the number of $CD8^+CD28^+$ T cell and T lymphocyte age. $CD8^+CD28^+$ T cell differentiate into the killer T cell that targets a virus and a cancer cell, the number of $CD28^+$ T cell decreases with age, as a result the number of $CD8^+CD28^+$ T cell is lowered. The T-lymphocyte age calculated from its numerical value with that is also rising (getting worse). In the present study, because the number of $CD8^+CD28^+$ T cell increased significantly in the EMXG ingestion group but not in the placebo group, so the T lymphocyte age, which represents the immunological age, decreased (getting better) by 0.8 years in the EMXG ingestion group after 12 weeks, on the other hand, is the T lymphocyte age aging in the placebo group, it can suppose the ingestion of EMXG is effective to maintain the state of aging or stress.

ECEM is an extract from culture of effective microorganisms, while photosynthetic bacteria (*Rhodopseudomonas palustris*) is a gram-negative bacteria among effective microorganism used for production of ECEM. Therefore, Lipopolysaccaride (LPS) of a cell wall ingredient of gram-negative bacteria is included in the ECEM. LPS is known having immunopotentiating actions. It is well-known that the LPS-elicited inflammatory response is initiated by binding of TLR4 with LPS on the cell surface, followed by activation of intracellular machinery, particularly the nuclear factor (NF)-kappaB signaling system[8]. About immunity activating effect of LPS, there are reported a lot that antiallergy action activating macrophage[9)10)], function as immunological adjuvants for antigen-specific immune responses[11)] and preventing lifestyle and allergic deseases[12)].

And also, ECEM of EMXG are reported that it has distinctive anti-inflammatory and immunostimulatory actions which are related to pro-inflammatory genes and natural killer T cells. In addition, it is known that ECEM contains anti-inflammatory compounds, including embelin, gingerol, diallyl sulfide, and 16-alpha-hydroxyestrone[3].

In consequence, LPS of ECEM is thought to trigger activation of the immune response in both inflammation and antigen presentation, and any components included in ECEM are thought to be important for these functions.

This suggests that the ingestion of EMXG improves the immunological status of human.

Although it is presumed that EMXG ingestion is effective on SEIV or antioxidation, we could not find the significant difference on this study. For exercising a definite effect on them, it should have been necessary to continuously ingest EMXG for a longer period of time. We need further study to scrutinize that point of view.

On the biochemical blood test, urine analysis, and medical examination we did not find any serious change of health contingent to the ingestion. Therefore it can be said that under the condition of this study, this EMXG was safe.

CONCLUSIONS

The objective of this study was to investigate the efficacy of EM・X GOLD (EMXG), which is a health drink containing extract from culture of effective microorganisms (ECEM) on human immunity. Of 47 applicants, 38 subjects completed the trial. SIV, the scoring system that can combine seven major immunological parameters, was used to evaluate immunological vigor. Among SIV, T lymphocyte age and $CD8^+CD28^+$ T cell showed significant improvement after the ingestion of EMXG for 12 weeks when compared to the placebo. These results suggest that the daily supplementation of EMXG can be effective to keep immunity function at a high level. No clinically significant adverse events or side effects occurred during this study. There was no safety concern in the study conditions.

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