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Evaluation of the toxicity and safety of the antioxidant beverage effective microorganisms-X (EM-X) in animal models

Bin Ke [a,**], Yun-Fei Liang [b], Zheng-Xian Zhong [c], Terou Higa [d], Okezie I. Aruoma [e,f,*]

[a] *EM Research Organization, 468 Kawasaki Uruma City Okinawa 904-2203, Japan*
[b] *Department of Physiology, School of Medicine, University of the Ryukyus, Okinawa 903-0215, Japan*
[c] *Division of Pharmacology, Guangxi Institute of Traditional Medical and Pharmaceutical sciences, Nanning, China*
[d] *Department of Horticulture, College of Agriculture, University of the Ryukyus, Okinawa 903-0213, Japan*
[e] *Faculty of Health and Social Care, London South Bank University, 103 Borough Road, London SE1 0AA, UK*
[f] *Department of Veterinary Public Health, College of Veterinary medicine, Seoul National University, San 56-1, Shillim-Dong, Kwanak-gu, Seoul 151-742, South Korea*

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Abstract

The acute and chronic toxicity tests and the mutagenic test of the extracts from the fermentation of plants with effective microorganisms (EM-X) were performed in the mouse and the rat. In acute toxicity test, mice were orally treated three times per day with 20-fold of concentrated EM-X for 7 days. For chronic toxicity test, the rats were orally treated with original EM-X once a day for 90 days at the dosages of 180, 120 or 60 ml/kg. At the levels tested EM-X did not lead to significant changes in food consumption, body weight, behaviors and stools. Hematological assays on red blood, white blood cell, hemoglobin, platelets, lymphocyte, granulocyte, middle cell and coagulation time and the biochemical assays on aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen, total protein, albumin, glucose, total bilirubin, creatinine and total cholesterol did not show abnormal changes. The histological inspection of principal organs of the heart, liver, spleen, lung and kidney did not show significant pathological changes. The delaying toxic reactions were detected 2 weeks after administration of EM-X was stopped. The mutagenic test showed that EM-X did not cause mutagenesis and tests of micronucleus of bone marrow cell and sperm shape abnormality upon EM-X were negative. The maximal tolerance dose of EM-X was calculated to be 1800 ml/kg BW in the mouse and rat. Thus, oral administration of EM-X does not present acute and chronic toxicity and mutagenic effects in the animals.

Keywords: EM-X; Antioxidants; Effective microorganisms; Fermentation products; Mutagenicity; Functional foods

1. Introduction

The beverage effective microorganisms-X (EM-X) is a novel antioxidant cocktail derived from fermentation of unpolished rice, papaya and sea weeds with grouped effective microorganisms of lactic acid bacteria, yeast and photosynthetic bacteria. It contains mixed-extracts of plants and effective microorganisms. EM-X contains over 40 minerals (antioxidants such as flavonoids, kaempferol, panaxin, quercetin, lycopene, oryzanolum, ascorbic acid, tocopherol, ubiquinone) and other bioactive substances such as nucleotide, peptide and aminoacid, such as nicotinamide mononucleotide, nicotinamide adenine dinucleotide, L-alanine and L-glutamine (Sato et al., 1997).

EM-X has been shown to enhance the activities of the human T and B lymphocytes and natural killer cell, possess anti-HIV activity, increase the serum superoxide dismutase, decrease the malondialdelhyde on the D-galactose-induced aging mice, possess antihyperglycemic effects and to prolong

* Corresponding author. Tel.: +44 20 7815 7927.
** Co-corresponding author. Fax: +81 98 972 6068.
E-mail addresses: kebin@emro.co.jp (B. Ke), aruomaoi@lsbu.ac.uk (O.I. Aruoma).

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the life of fruit fly (Ke et al., 2001). EM-X inhibits the TNF-α and oxidant-induced interleukin-8 release in epithelial A543 cells and the peroxidation of phospholipids (Deiana et al., 2002) and modulates oxidative damage in the kidney and liver of rats by protecting unsaturated fatty acids (Aruoma et al., 2002). The ability of the antioxidant beverage to modulate neuronal cell death due to excitotoxicity has been documented (Aruoma et al., 2003). The loss of tyrosine hydroxylase positive cells in the substantia nigra pars compacta following the treatment of rats with the neurotoxin 6-hydroxydopamine has been shown to significantly attenuated by EM-X, further suggesting the neuroprotective potential of the beverage (Datla et al., 2004). The potential use of EM-X in the management of breast cancer patients has been suggested by Usmani et al. (2000). EM-X is widely available in Asia as a health beverage and there is the prospect of continued acceptance in clinical practice. Given this outcome, the aim of this study was to examine the acute, chronic and mutagenic toxicities of oral treatment of EM-X in animal models.

2. Materials and methods

2.1. Materials

NIH mice of both sexes weighing 20 ± 2 g were used in the acute toxicity test and those weighing 25–32 g were used for the mutagenic test. SD rats of both sexes weighing 110–130 g were used in the acute toxicity test. The animals were sex-separately housed in the air-conditioned animal rooms at temperature of $22 \pm 2\,^{\circ}C$ and relative humidity of $60 \pm 5\%$. Foods and water were allowed ad libitum. In the mutagenic tests, the S. Typhimuriums of TA97, TA98, TA100 and TA102, which were carefully assayed to fit the demand gene types were used as testing strains in Ames tests. All testing strains were cultured over night and the bacterial concentrations were $\geq 10^{12}\,l^{-1}$. S-9 was prepared from liver homogenate of rats induced by Aroclor 1254. The concentration of S-9 in the mixture that was composed of S-9 and assistant factors was 10% and S-9 protein contained in the mixture was 38 g/l. Dexon, sodium azide (NaN_3) and 2-aminofluorene (2AF) were used in Ames test as positive control agents and cyclophosphane was used in the test of marrow micronucleus and sperm shape assay. The original EM-X was obtained from EM Research Organization (Okinawa, Japan). Abbott cell-byn (mode 1600, Abbott, America) and Hitachi chemistry analyzer (model 7170, Hitachi, Japan) were used in the chronic toxicity test.

2.2. Toxicity tests

Twenty mice were used in the acute toxicity test. EM-X was concentrated into a 20-fold liquid from the original EM-X. The mice were orally treated with the concentrate three times per day at the volume of 0.3 ml/10 g BW. The daily dose was 90 ml/kg BW. Changes in body weight, food consumption pattern, behaviors and deaths were observed over 7 days. For the chronic toxicity test, 80 rats were equally divided into three EM-X dose groups of 180, 120 and 60 ml/kg and a control group. The rats of EM-X groups were orally treated with EM-X concentrates at the volume of 1.2 ml/kg once a day for 12 weeks. The EM-X concentrates were 150-, 100- and 50-fold of the daily dose used in clinic for the high-, medium- and low-dose groups, respectively. The control group was treated with the same volume of distilled water. The general habits of the states of the rats were observed daily. Changes in body weights were noted and the dosage of EM-X adjusted accordingly. Twenty-four hours after last administration of tested agents, 12 rats including both sexes in each group were killed and blood samples were collected. The hematological assays on red blood count (RBC), white blood-cell count (WBC), hemoglobin (Hb), blood platelets count (BPC), lymphocyte (L), granulocyte (GRAN), middle cell (MID) and coagulation time (CT) were performed using Abbott cell-byn and the biochemical assays on the serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), total protein (TP), albumin (ALB), glucose (GLU), total bilirubin (T-BIL), creatinine (Crea) and total cholesterol (T-CHO) were performed using Hitachi chemistry analyzer. Physiological and histological examinations of the organs were carried out after dissection. The heart, liver, spleen, lung, kidney, renicapsule, thymus, testis, ovary and pancreas were removed, weighed and their organ coefficients assessed. The organs were then fixed with 10% formalin solution for 48 h for pathological examinations. Pathological sections were made with conventional methods and examined under light microscopy. The remaining rats were raised for a further 2 weeks at which point the administration of EM-X was stopped. The animals were sacrificed and the relevant tissues were used for the various assays.

In mutagenic test, EM-X was prepared into five concentrations of 5000, 500, 50, 5 and 0.2 μg/disc with distilled water and treated with high-pressure sterilization for Ames tests. The stated dilutions of EM-X were used for the micronucleus test of bone marrow cell and sperm shape abnormality test on mice. Four testing strains, e.g. TA97, TA98, TA100 and TA102 of S. Typhimurium mutation types were used for Ames test. S-9 mixture was used for activating metabolic system in vitro. Growing bacterium solution of 0.1 ml and testing solution of 0.1 ml were added onto the upper layer of agar and S-9 mixtures were put on lower layer of culture medium plate. When metabolisms were activated S-9 mixture of 0.5 ml were added once again. Five doses of EM-X of 5000, 500, 50, 5 and 0.2 μg/disc, spontaneous reversion, positive mutagen and non-bacterium control were designed in Ames test. The numbers of reverse colony in each disc were counted after cultivated for 48 h at $37\,^{\circ}C$. When the rate of the increase in the reverse colony numbers exceeded that of the spontaneous reversion rate, the responses are considered positive if the effects are