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Radioprotection effect and Immune Activity for Enterococcus Faecalis (EF 2001)
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Key word: Enterococcus Fekaris 2001 (EF 2001), Math/A fibrosarcome, analgesic, anti-neoplasm effects

ABSTRACT
The preparation reduced to powder, which removed fungi mainly from EF 2001, which was EF 2001, was used in this study. Because L/P activity was recognized from EF 2001, it was examined about the anti-neoplasm effects that it faces Math/A fibrosarcome in the mice, and the analgesic action by the preceding research. They are inoculated 1 × 10⁶ cells and 2 × 10⁵ directly in the right and the left low abdomen region the intratumorale as for the anti-neoplasm simulation by the way of the administration meth A fibrosarcome to each of the BALB/c mouse. The dimension of the tumor enhancement was measured more than the implantation posticus the 3rd day from a total 10 times, the top of the cutaneous even in the 2nd de die in diem every other day, and the total was examined, and gradation was seen at the time as the sutra. It was dissolved with the fraction of 1mg/0.1ml/mouse in the isotonic sodium chloride solution, and specimen substantial administered neoplasms vicinal rear 3, 4, the 5th-day total 3 times for the right intratumorale. And, pain relief simulation was done with the acetic acid lasing method. The L/P activity had statistical significant difference with the 200ug/mouse dose rate (p<0.05). Catastaltic determination was judged by the antitumor action the tumor enhancement in both low abdominal regions. Then, catastaltic determination was recognized with 1000mg/kg by the analgesic action to the control group.

INTRODUCTION
EF 2001 likes a rich place in food and the nutrient such as an intraintestinal, and lives. Therefore, it is the typical Bacterium group, which coexists with the relations, which are close to our dietary life and the life circle kept. Anciently, humans have used EF 2001 skillfully. Bean paste, a traditionally fermented food like the pickles and yogurt, a fermented milk preparation like a cheese are the typical examples. EF 2001 as a food is taken together with the fermented food. Recently, it was together wide with the verification of the functionality, and it was used for the food processing as well, and relations with the well being of the human were argued large. It has the medicament
reason effects of the EF 2001 reported the antitumor action, the cancerous prophylactics function, the immunization activity function, the interferon enhancement, the blood pressure descent function, the mutagenicity material ablation function, and so on (1,2,3,4). Lactic acid Cocccaeae (EF 2001 stock; The following EF2001 abbreviation) was cultivated, and Enterococcus / Fekaris 2001 preparation mixed the EF2001, which contains EF 2001 production material, and constituents. After that, the grain movement panicles granulator vessel manufactured it, and it got preparation EF 2001 (5,6). Freeze drying made it do the water-soluble component of the EF 2001, and got the fraction which contained water bacteriolysis constituent in this study. This reduced to powder was used as material, and antitumor action against the leucocyte (lymphocyte) increase function of the mice; the Math-A fibrosarcoma cancerous of the mice was examined. And, it was examined about the analgesic action by the way of the acetic acid Writthing of the mice.

MATERIAL AND METHODS
EF 2001 was used in this study. After aqua distillate 200 ml was added to EF 2001 20g and stirred, it was put for 2 hours by the room temperature as a preparation of the water extracts EF 2001. After that, 30min centrifugal separation was done with 1000rpm, and freeze-drying did clear supernatant liquid. Water extracts EF 2001 was made the material of the physiology activity of investigation. We used the new live birth of the brood of the Swiss-Webster mouse within from12 to 24 hours old for the simulation. The simulation procedure of the leucocyte (lymphocyte) increase function went by using the procedure that Hands made Metcalf's' procedure strange (7,8). Isotonic sodium chloride solution was administered in the control group, and intraperitoneal administration did water extracts EF 2001 (200ug/mouse) in the specimen group. Blood was drawn from the caudal vein before the administration in the administration posticus 6 days, 10 days, the 14th day, and determination made gracillis panicles blood film. It dyed in the Wright staining technique, and lymphocyte and leucocyte were counted by using the microscope (magnification 10x40 times) with mechanical stage. The ratio (L/P ratio to the leukocyte) of the number of the lymphocyte was calculated. The decision of the effect went by the t-test. The BALB/c maleness mouse was used age of six weeks as an animal used for experiment of the antitumor action. Simulation was done in accordance with Ebins' "double-grafted tumor system" (9). 1x 10^6 and 2x 10^5 Math-A fibrosarcoma cellularis were transplanted to each of the right of the normalize mice and the left abdomen region. The fifth-day total administered isotonic sodium chloride solution for three days and for four days three times in the control group the neoplastic cell implantation posticos. Water extracts EF 2001 (1 mg/0.1ml/mouse) was administered directly in the specimen group for the intratumorale of the right low abdomen region. As for the determination, the head sutra of the neoplasm and short sutra were measured with the micrometer caliper from the neoplasm implantation posticus the 3rd day from a total 12 times, the top of the cutaneous until the 25th day at the every other day. The total was examined, and gradation was asked at the time as the sutra of the tumor enhancement. Neoplasm was taken out for the neoplasm implantation posticus the 25th day, and ponderis was measured. The decision of the effect went by the t-test. After it fasted for four hours, the ddy male mice was used age of five weeks as an animal used for experiment of the analgesis action. The acetic acid liquid of the algescis substance was dissolved in the disinfections isotonic sodium chloride solution,
and used as 0.7% (W/V) solution. It went in accordance with winters' procedure (10). Aqua distillate (10 mg/kg, B.W.) was administered in the control group, and water extracts EF 2001 (1000 mg/kg, B.W.) was administered in the specimen group. Internal use did Aspirin aluminum (500 mg/kg, B.W.) in the Positive-control group. Intraperitoneal administration (10 mg/kg, B.W.) did acetic acid isotonic sodium chloride solution of 0.7% after 1 hour, and the number of times of 10-minute Writhing was counted after 10 minutes. The analgesic effect of material examined depression effect with the control group. The decision of the statistical significant went by the t-test.

RESULTS
Fructification against the increase function of the leucocyte (lymphocyte) ratio is shown to Table1. The L/P ratio of the administration group became the administration post the 6th day in 0.65 +/- 0.112, the 10th day in 0.94 +/- 2.051 and the 14th day in 3.69 +/- 0.086. It had 1% and the statistical significant difference 5% by the critical rate for 6 days in the 10th day in the 14th day to the control. The fructification of the antitumor action simulation against the cancerous of Meth A-fibrosarcome is shown is to Table2, Table3, and Table4. As for the anatomic fructification, which went after the neoplasm implantation in the 25th day, the neoplasm ponderis of the right hypogastric region was by the control group in 1.468 +/- 0.521. Well the neoplasm elimination of 1 example was seen to this specimen administration group in 0.640 +/- 0.211. But, catastrophic wasn't recognized as the neoplasm ponderis of the left abdomen region. Next, the neoplasm of the right abdomen region became with the specimen administration group by the target gradation from the 3rd day at the time as the sutra of the 25th-day tumor enhancement in the 2nd day in 137.3 +/- 29.2 mm², the 23rd day in 131.3 +/- 25.3 mm², the 25th day in 127.9 +/- 33.0 mm². Therefore, obviously it had statistical significant difference to the control group. But, some catastrophic wasn't recognized as the neoplasm of the left abdomen region between the specimen administration group and the control group. Analgesic action is shown in the following. The number of Writhing of the control group was 33.0 +/- 4.0. It was 8.0 +/- 3.8 by the number of Writhing of the specimen administration group to this. It was 15.9 +/- 4.6 by the number of Writhing of the Aspirin administration group. Therefore, an Aspirin administration group recognized some catastrophic 1% of the critical rate with the administration group 5% critical rate.

CONCLUSION
The L/P ratio of the administration group was compared with the control group after the administration by the 14th day, and statistical significant difference was clear as a result of the increase function to the leucocyte (lymphocyte) (P<0.01).
Lactate is released in large quantity from sites of sepsis and inflammation (11). Haji-Michaels asked whether the increased lactate production found in sepsis could be explained by the augmented glycolysis of inflammatory cells. The glycolytic metabolism of rat peritoneal leukocytes was measured following cecal ligation and perforation (CLP) or sham laparotomy. CLP augmented glucose uptake, the pentose phosphate pathway, and glucose oxidation. Lactate output increased from 1.03 +/- 0.05 to 1.20 +/- 0.05 fmol x cell (-1) x min (-1) (P < .001). Total lactate output of peritoneal lavage fluid increased from 7.94 +/- 2.59 to 28.12 +/- 5.60 n mol L x min (-1) (P < .005). The effect of lipopolysaccharide (LPS) on the lactate output of whole blood from 31
critically ill patients was measured. Leukocyte lactate production was calculated by multiple linear regression analysis. This rate of production is so high that it suggests that their different cell populations and state of activity may affect the lactate output of different tissue beds in sepsis. This study supports the hypothesis that lactate may be more a product of inflammation than a marker of tissue hypoxia in sepsis (12).

In Simoes study, HG The equilibrium point between blood lactate production and removal (La- (min)) and the individual anaerobic threshold (IAT) protocols have been used to evaluate exercise. Then, capillary blood (25 micro liter) was collected for [La-] b and [Glc] b measurements. The IAT and IGT were determined by [La-]b and [Glc]b kinetics during the second test. The La-(min) and Glc(min) were determined considering the lowest [La-] and [Glc]b during the third test. No differences were observed (P < 0.05) and high correlations were obtained between the velocities at IAT. Therefore, Simoes concluded that for these subjects it was possible to evaluate the aerobic capacity by IGT and Glc (min) as well as by IAT and La- (min) (13).

As a result of the antitumor action against the cancerous of Meth/A-fibrosarcome, The neoplasm ponderis of the right abdomen region was compared with the control group after the neoplasm implantation in the 25th day, and the neoplasm elimination example of one example was seen with the specimen administration group. But, statistical catastrophic wasn't recognized as the neoplasm ponderis of the left abdomen region. The gradation was compared with the control group in the neoplasm of the right abdomen region from the 3rd day at the time as the sutra of the 25th-day tumor enhancement, and statistical significant difference was clear next with the administration group from the 2nd de die in diem (P<0.05).

In Battelinos reports, tumor necrosis factor-alpha (TNF-alpha), an important mediator of endotoxic shock, induces hypoglycemia and shock in adult animals. Therefore, indomethacin ameliorates TNF-alpha-induced hypoglycemia in the adult. However, effects of TNF-alpha on glucose metabolism in the newborn have not been well documented (14).

The present study showed that in 10-day-old rats injected with TNF-alpha (4.5 x 10(7) U/kg, intraperitoneally) the plasma glucose concentration increased from 4.1 +/- 0.3 mmol/L to 6.9 +/- 0.5 mmol/L (P < .05) at 2 hours and subsequently decreased to 1.4 +/- 0.5 mmol/L (P < .05) at 6 hours, although plasma lactate concentration increased from 1.1 +/- 0.1 mmol/L to 5.5 +/- 0.3 mmol/L (P < .05) at 6 hours. Plasma insulin concentration remained unchanged throughout the experiment. Glucose uptake increased in association with the increase of GLUT1 mRNA abundance. TNF-alpha decreased mRNA abundance of GLUT 2 and phosphoenolpyruvate carboxykinase (PEPCK) in liver, suggesting decreased gluconeogenesis. Battelinos concluded that TNF-alpha induced hypoglycemia, increasing GLUT1 mRNA abundance and glucose uptake and decreasing PEPCK mRNA abundance in 10-day-old rats (15).

Then, Battelinos concluded that TNF-alpha induced hypoglycemia, increasing GLUT1 mRNA abundance and glucose uptake and decreasing PEPCK mRNA abundance in 10-day-old rats (15).

In Arakis study, the survival time of the tumor-bearing mice was prolonged in the CDDP-MC group, but not in the group treated with cisplatin solution. By using this
modified formulation of cisplatin, the toxicity of the drug can be reduced, and effective concentrations of the drug can be maintained locally for prolonged periods of time (16). Tokudasa recognized the innidation catastrophic of peritoneal metastasis by using poly-D, L-lactic acid and polyethylene glycol acid. Therefore, the survival of rats with peritoneal metastasis was better in the CDDP-PPMS group than in the CDDP-SOL group (17).

But, statistical catastrophic wasn't recognized as the neoplasm of the left abdomen region between the specimen administration group and the control group in this research. It thinks with the thing that water extracts EF 2001 functioned directly in the neoplasm of the right abdomen region from this fructification for the hyperplastic catalatstic of the neoplastic cell by some function institution.

And, there was no difference to the neoplasm of the left abdomen region. But, because catastrophic determination was judged, EF 2001 of the water exhaustion is thought T-cellularis macrophage potentiation involve. And, recipient interstitial is thought about because it is the fructification which immunization activity was (18).

Cell survival, cellular damage, and antagonistic activity were investigated after spray drying of four bacteriocin-producing strains of lactic acid bacteria in Mauriellos study. Trials were performed with bacteria suspended in skimmed milk or directly grown in whey.

Three air temperatures at the inlet of the drier (160, 180, and 200 degrees C) and three flow rates (10, 13, and 17 ml/min) were assayed. During storage for 2 months at 4 degrees C, all samples, but mainly the lactococcal strains, displayed a gradual decrease in cell survival.

Bacteriocin activity remained at the same level, allowing powders to be considered as effective biopreservatives (19).

Statistical significant difference was clear with the EF 2001 administration group of the water exhaustion in comparison with the control group as a result of the analgesic action simulation (P<0.01). Aspirin is thought acid non-steroid anti-inflammatory and analgesic action synthesis inhibition Prostaglandin show. EF 2001 of the water exhaustion may be probably based on the obliteration of the production of the algesic substance, disengagement, catastrophic or the path of pain and others.

REFERENCES
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Material & Methods

- Anti-cancer effects
  - Seven-weeks-old male ICR (Crj) mice
- Cancer cells: sarcoma 180 ($2 \times 10^6$)
- EF 2001 5mg/kg of heat-killed EF2001 (EF2001) were injected interpretational(endoceiliac) each for 2 weeks every other day
- Statistical methods: t-test
Enterococcus Faecalis under microscope (left) × 20, (right) × 12900
Materials and Methods

1. Radiation protection effects

Seven-weeks-old male C3H mice
12mg/Kg 24mg/Kg of heat-killed EF2001 (EF2001) were injected interpretational each for 2 weeks every other day

8Gy of whole body irradiation (Philips co. 200kV)

Change of body weight Survival after irradiation

Sections of the large and small intestines with a microscope
2. Assay of NK cell activity by $^{51}$Cr label YAC-1 cells

Injection of the EF2001, in the same condition using the examination of radiation protection effect.

The mice was scarified and the spleen was extracted. The spleen was smashed with stainless steel mesh, and then mixed PBS and the suspect ion was centrifuged three times.

The concentration of the spleen cells was adjusted to be $2 \times 10^7$ cells/ml. Then, $1.25 \times 10^6$, $2.5 \times 10^6$, $5.0 \times 10^6$, $10.0 \times 10^6$, $20.0 \times 10^6$ of the spleen cells were added to $1 \times 10^4$ of YAC-1 cell which labeled $^{51}$Cr of 1 mCi and incubated 96 hole plate for 6 hours.

Only liquid component in each hole measured with liquid scintillation.
Survival after irradiation
Surviving fraction was increased after injection of EF2001.
Small intestines

Control

8Gy + 12mg/kg

8Gy + 24mg/kg
NK cells activity
Activities of NK cells are enhanced 1.46 and 1.94 times in EF200112mg and EF200124mg groups respectively.
Anti-tumor effect of BRM on Meth A fibrosarcoma (solid type) in BALB/c mice

* p<0.05 vs Control
Anti-tumor effect of EF-2001 on S-180 in ICR mice

**P<0.01  *P<0.05 vs Control group**
BLB/C mice of IgE in the blood. Each histogram represents the mean value ±SE for 10 mice IgE (M). Significantly different \( *p<0.05 \) Control vs. EF 2001.
Fig. 2. C3H mice of IgM in the blood. Each histogram represents the mean value ±SE for 10 mice IgM (M) Significantly different *p<0.05 Control vs. EF 2001.
Fig. 3. C3H mice of IgG in the blood. Each histogram represents the mean value ±SE for 10 mice IgG (M). Significantly different *p<0.05 Control vs. EF 2001.
Conclusion 1

- Anti-cancer effects: EF 2001 administration group: positive
- EF 2001 to the radiation protection effect: precision
- Immune activity effect: EF 2001 administration group: positive
- Anti-aging effect: EF 2001 administration group: positive
- Long life effect: EF 2001 administration group: positive
- It put the Enterococcus Faecalis dosage, and the level of total IgE in serum glutamic-oxaloacetic fell.
- The level of total IgM in serum glutamic-oxaloacetic increased the Enterococcus Faecalis dosage group. However, the level of total IgG in serum glutamic-oxaloacetic rather fell slightly.
Conclusion 2

1. It let cell-mediated immunity such as a macrophage and natural killer T cell activate, and the immunization activation action that Enterococcus Faecalis has does not become it, and promotion of humeral immunity anti-action is thought about.

2. In the dosage of Enterococcus Faecalis, an IL-2 level in blood rises, and it think that cytokine of the spleen changed from Th2 type into Th1 type. Therefore, it decrease IgG and an IgE level, and it is suggested that it showed an allergic restraint effect.