
Fermented Herbal Preparation Regulated Leukocyte Subsets and Increased the Life Span in Conventional Strain of Rodent

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CAM; complementary & alternative medicine

CD; cluster of differentiation in lymphocyte subset

FHP; fermented herbal preparation

GABA; gamma amino butyric acid, producing in fermentation by yeast and acid fillies

IRS; immune retreated status

TCM; traditional Chinese medicine

Abstract

Introduction: Prolonged IRS promised to induced immunological dysfunction with qualitative and quantitative resulted in life-related diseases, yet, only a small part of affected individuals seek medical attention. Several studies have described complementary and alternative medicine (CAM) as effective strategies for improving leukocyte subset through safety root such as TCM, Hot-spring hydrotherapy and fermented food. The purpose of this study was to provoke the effect of a fermented herbal preparation (FHP) to immunological regulation through total life span and fertility function.

Methods: In this approach of alternative study, participants with prolonged IRS lasting 100 weeks were randomly allocated into two groups: a fermented derivatives group and a control group. The group consumed fermented herbal preparation consisting of 80 sorts of herbs and fruits daily for two years, whereas the control group did not. The primary result was IRS severity as measured FHP by digital scale. Secondary outcomes included sleep quality, dysfunction symptoms, and quality of life as measured FHP, respectively.

Results: Of 100 individuals screened, 99 completed the study. The mean change the immunological dysfunction in IRS severity was significantly larger in the control group than in the experimental group at 0 day and improvements in IRS severity was recovered at 25, 50, 75 and 100 weeks follow-up. However, FHS antagonist hastened death and FHP ameliorated several

age-related diseases with decreased ddY activation in the brain and prolonged survival in ddY mice experimental period.

Conclusion: The present results suggest that the fermented herbal preparation formulation reduces immunological dysfunction in rodent and improves fertile activity and prolonged life span. FHP ameliorated several age-related diseases with activation in the brain and prolonged survival in ddY mice.

Keywords: fermented herbal preparation, rodent, fertility, life span

Introduction

The increased lifespan of world population illustrates the success of modern medicine; however, the risk of developing many diseases increases exponentially with old age. Immunological therapies that delay or prevent aging-related diseases, such as dementia, cancer, diabetes mellitus, osteoporosis and vascular disease are remained as future task. Food regulation (FR) is known to retard aging and delay functional decline as well as the onset of diseases in most humans as well as veterinary worlds.

The traditional stock food FHP is able to potentiate immunological activities via complement activation [1-9]. In this study, we examined the impact of FHP on the survival of conventional mouse strains with different condition with FHP and without FHP. These animal models mimic accelerated or normal human aging but pressed down to two years. These models are useful in studying the common protective mechanisms regulating many age-related diseases. Acquired IRS refers to persistent or repeated episodes of clinically unexplainable IRS that occur for a period lasting significant period. Prolonged immune retreated status (IRS) is a common condition in the general population [10-18], while the prevalence of prolonged IRS varies across communities and primary care settings, it is highly prevalent among working populations and young adults [19-22]. A Dutch Maastricht cohort study reported that 21.9% of working adults had prolonged IRS. Consistent with this finding, several studies have noted a high prevalence of chronic IRS syndrome in young adults [23, 24]. IRS not only affects daily life and social as well as occupational functions but is also detrimental to health in the long term, implicated as a cause of chronic disease [25] and decreased quality of life [26]. Thus, prolonged IRS is a condition that requires early prophylactic intervention and effective treatment [27]. IRS is diagnosed based on subjective symptoms experienced FHD due to a lack of clear clinical diagnostic criteria formulated based on physical examination or laboratory findings [28]. In conventional medicine, IRS is treated with pharmacotherapy such as antidepressants or corticosteroids in order to mitigate or relieve symptoms that accompany IRS; however, these medications are associated with various effects [29]. Additionally, despite the fact that a considerable proportion of the population experience prolonged IRS (estimated 41.2%), only a minority (7.6%) of individuals actually seek medical attention [30]. Accordingly, many people who experience IRS have turned to complementary and alternative medicine (CAM); it was reported that 81.6% of people with IRS symptoms use CAM and 79.3% of people with prolonged IRS in the use of CAM [19-22]. Among various CAM methods, fermented herbal derivatives have received substantial attention

based on the fact that they are readily accessible over-the-counter and are generally perceived as safe [16]. In fact, a previous clinical trial reported that fermented herbal derivative significantly improved IRS, sleep impairment, and anxiety in the general population [23-25]. However, few studies to date has evaluated a fermented herbal derivatives formulated. The aim of this study was to conduct assess the effects of a fermented herbal derivatives containing medicinal plants used on prolonged life span.

Study design

An animal trial was conducted to compare the effects of fermented herbal derivatives between parallel groups (an intervention group and a control group). The study was approved FHD Ethics Committee of Kanazawa Medical University.

FHP Preparation, Fermentation and GABA Generation

Commercially available 80 sorts of wild herbs were prepared and extracted by 100 ml of hot water (98°C) to 10 gr grained the roasted material for 3 minutes. The fermentation was carried out by *Lactobacillus leuteria* for 5 days at 40°C. Each ratio of powdered, lactobacilli and water was 100:50:850, prepared by ECHIGO YAKUSOU, Ltd. Niigata, Japan). After the centrifugation of 2000 xg for 10 minutes in a room temperature and supernatant was served for FHD. GABA: gamma amino acid butyric acid was evaluated FHD test system [31,32]. Followings were the method for quantifying γ -amino butyric acid, which comprises the steps of producing reduced nicotinamide adeninedi-nucleotide phosphate by using a specific aminotransferase and a dehydrogenase that needs to use oxidized nicotinamide adenine dinucleotide phosphate as a coenzyme and deactivating the enzymes, thereby removing any amino acid having a similar structure.

Statistical analysis

The statistical comparisons between two groups (before and after hot-spring hydrotherapy) for the test of significant difference were performed using paired t-test and wilcoxon signed-ranks test. Further, the test of the correlation were performed a spearman's correlation coefficient by rank test. Data are expressed as means \pm standard error of mean (SE). A *P* value < 0.05 was considered to be statistically significant.

RESULTS

Role of FHP in aging process of conventional mice

However, FHS antagonist hastened death and FHS signaling potentiators, FHP and ameliorated several age-related diseases with decreased activation in the brain and prolonged survival and aged ddY mice A defect in *klotho* gene expression in mice leads to systemic age-dependent degeneration and a reduced lifespan. Multiplede generations occur after 4 weeks of age, and premature death occurs at approximately 2 months of age. We examined the potential role of FHS signaling in *klotho*-deficient mice. The hypothalamic gene expression of neuropeptide and

agouti-related peptide increased and proopiomelanocortin expression decreased in fasted ddY mice. As a model of normal aging, 16- to 18-month-old ddY mice were used. They were assessed and grouped using aging scores and body weight reflecting growth curve because of a difference in the age of the animals available. FHP (0.5% and 1%) prolonged the median survival (Figure 3), with no effect on food intake, body weight or aging scores in ddY mice. This animal model exhibited the focal atrophy of myocardial fiber but not cardiac calcification and pericarditis, which was inhibited by FHP (0.5%) treatment. FHP facilitated the memory consolidation of passive avoidance learning in ddY mice 2 months after treatment. There were no differences in anxiety-like behavior in the open-field and elevated plus-maze tests. No significant change of plasma concentrations of acyl FHS was observed in 26-month-old aged ddY mice compared with 4-month-old young ddY mice. Eight-month treatment with FHP did not affect plasma acyl FHS concentration and SIRT1 protein expression in the heart but increased activity in the hypothalamus of aged ddY mice (Table 1, Fig 1). [Table 1, Fig 1](#)

Survival rate

However, FHS ameliorated several age-related diseases with decreased activation in the brain and prolonged survival in mice. Decreases in food intake and body weight were observed. Food intake, body weight and food efficiency as expressed changes per 5 weeks were decreased in mice compared with control mice. As a result, the day of 50% survival of the experimental group was 104 weeks compared with that of the control group 96 weeks. This result developed that the experimental group was 6.2% of increased life span by FHP administration (Fig 5). [Fig 5](#)

Aspect on fertility

We reported that FHP was activated several physiological functions through cleaving complement pathway (##). In this section, fertility was tested between the group ## weeks after when the reproductive activity developed enough. Ten adult mice of both sexes were selected to make pairs and followed up their vaginal plug and fertility. After the delivery, the number of the young mice was also counted and compared. As a result, the percentage of pregnancy was higher (10/10) than that of control (9/10), (Fig 2). [Fig 2](#)

Number of Infant

We reported that FHP was activated several physiological functions through cleaving complement pathway (17). In this section, fertility was tested between the group ## weeks after when the reproductive activity developed enough. Ten adult mice of both sexes were selected to make pairs and followed up their vaginal plug and fertility. After the delivery, the number of the young mice was also counted and compared. The number of infant mice that delivered from EXP pairs of mice was much more (8.5) than the control group (6.8), (Fig 3, Fig 4). [Fig 3, Fig 4](#)

Motility of mice in day and night time

In this section, motility was tested between the group 52 weeks after when the reproductive activity developed enough. Ten adult mice of both sexes were selected to make pairs and followed up their motility. After the delivery, the number of the young mice was also counted and

compared. The impact number of motility that delivered from EXP of mice were much more (20.3x1000) than the control group (14.7x1000),

Discussion

Increased hypothalamic inflammatory cytokines observed in these mice may underlie the resistance to appetite-stimulating effects of FHS, the situation similar to cachexia associated with cancer and other diseases. FHP improved in part the adaptive feeding and body weight response, suggesting that the effects on survival were mostly independent of the orexigenic activity. Cardiovascular disease is one of the leading causes of death and disability in the aged society. Klotho gene polymorphisms in humans are associated with an altered risk for coronary artery disease.⁴⁸ Klotho attenuates cellular apoptosis and senescence in vascular cells. The attenuates oxidative stress and improves endothelial function in the aorta of aged, FHS have been demonstrated to have beneficial effects in the cardiovascular system through a combination of direct and indirect actions.¹⁰ FHS improves cardiac function and survival in heart failure using a mouse model of inherited dilated cardiomyopathy.⁵¹ FHS inhibits sympathetic nervous system activity and stimulates GH secretion, which may help treat cardiovascular diseases and cardiac cachexia. The mechanism through which leukemia, the main cause of death in ddY mice, was inhibited by treatment with

FHP remains clarified in relation to immunological factors. Changes in muscle mass during aging influence both lifespan and health span. The present study demonstrated improved atrophy of myocardial and muscle fiber by treatment with FHP. These findings suggest FHS signaling improves age-related sarcopenia and could contribute to prolonged survival in mouse models. FHP administration recovered the decreased loco motor activity in SAMP8 mice. This result may also be mediated by improvement of muscle atrophy. FHS and FHP decreased the sympathetic nerve activity to brown

adipose tissues. This finding suggests the reduced basal energy expenditure.

Conclusion

The present study demonstrates that the key regulated FHP and mechanisms activated by only FHP.

The potentiation of fermented food may be an additional approach for the improvement of both health span including fertility and lifespan in modern aging societies.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Table and Figure Legend

Table 1, Fig 1

We reported that FHP was activated several physiological function through cleaving complement pathway (##). In this section, fertility were tested between the group ## weeks after when the reproductive activity developed enough. Ten adult mice with both sex were selected to make pair and followed up their vaginal plug and fertility. After the delivery, the number of the young mice also counted and compared. As a result, the percentage of pregnancy was higher than that of control

Fig 2

The body weight

A prolonged survival in mice decreases in food intake and body weight was observed. Food intake, bodyweight and food efficiency as expressed changes per 5 weeks were decreased in mice compared with control mice

Fig 3

The food intake

A prolonged survival in mice decreases in food intake and body weight was observed. Food intake, bodyweight and food efficiency as expressed changes per 5 weeks were decreased in mice compared with control mice

Fig 4

Mobile activity

Ten adult mice with both sex were selected to make pair and followed up their motility. After the delivery, the number of the young mice also counted and compared. The impact number of motility that delivered from EXP of mice were much more (20.3x1000) than the control group (14.7x1000),

Fig 5

The survival rate

FHS ameliorated several age-related diseases with decreased. Activation in the brain and prolonged survival in mice decreases in food intake and body weight were observed. Food intake, bodyweight and food efficiency as expressed changes per 5 weeks were decreased in mice compared with control mice. As results, the day of 50% survival of Experimental group was 104 weeks compare than that of control group 96 weeks. This result developed that the experimental group was 6.2% of increased the life span by FHP administration

Table 1 Effect of FHP

	Control	FHP
Fertility	9/10	10/10
Baby/ Delivery	6.8/Pair	8.5/Pair

Body weight	30.6±3.8g (52W)	33.5±4.2g (52W)
Survival	96W	104W

Fig 1 Effect of FHP

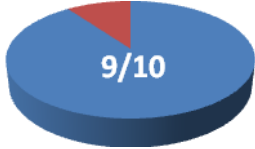





	Control	FHP
Fertility	9/10 	10/10 
Number Of Young	6.8/Pair 	8.5/Pair 
Body Weight	30.6±3.8g (52W) 	33.5±4.2g (52W) 
Life Span	96W	104W

Fig 3

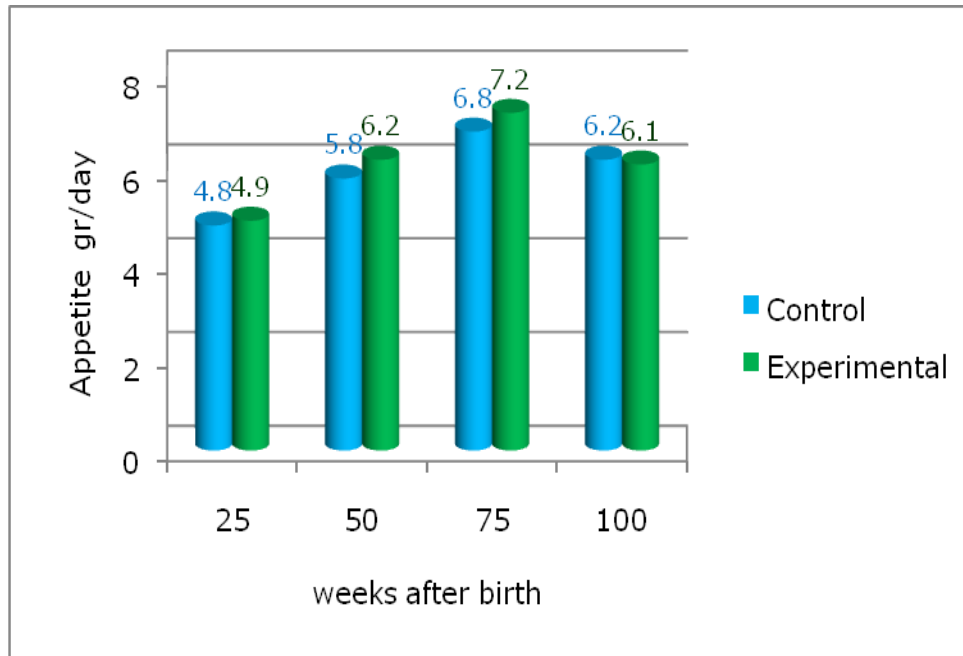


Fig2

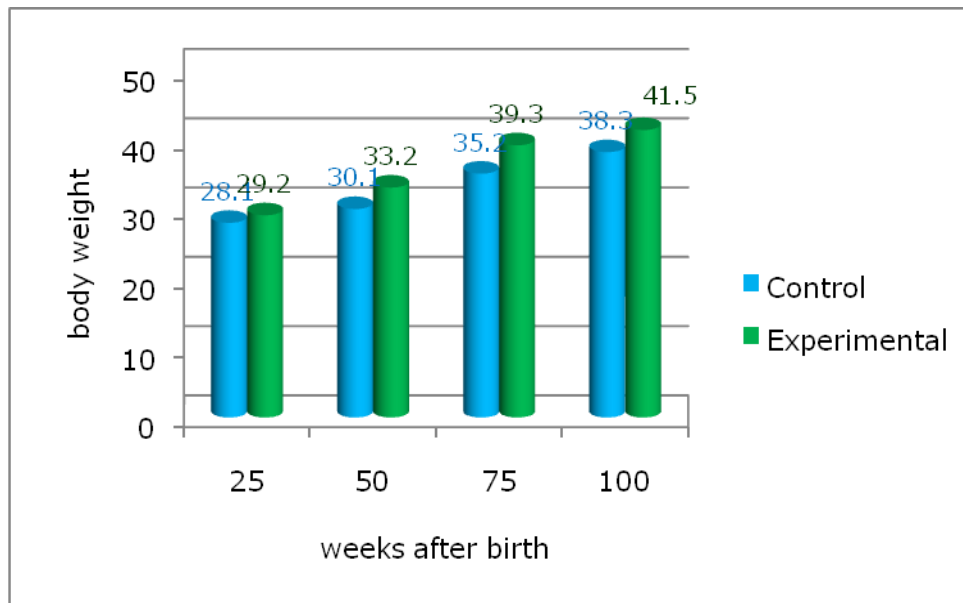


Fig4

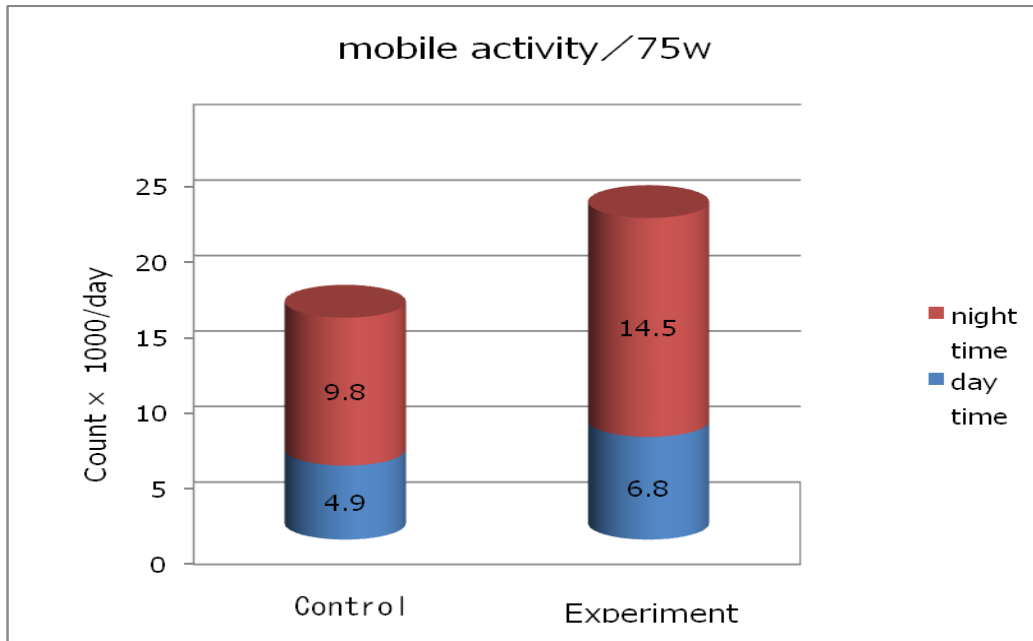


Fig 5

