International Scholars Journals

International Journal of Biochemistry and Biotechnology ISSN 2169-3048 Vol. 6 (4), pp. 767-774, April, 2017. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Effect of probiotic fermented soy milk and gamma radiation on nitrosourea-induced mammary carcinogenesis

[1]Omayma A Ragab*, [2]El-sonbaty SM, [1]Aziza SA and [1]Aboelftouh AE

[1]Biochemistry department, Faculty of Veterinary Medicine, Benha University, [2]Microbiology, National Centre of Radiation Research and Technology, Atomic Energy Authority.

Accepted 02 March, 2014

Functional food enters the concept of considering food not only as a necessity for living but also as a source of mental and physical well-being. The anti-proliferative effects of fermented soy suggested the need of low dose of gamma radiation in order to achieve an optimal therapeutic effect. This study investigated the potential chemotherapeutic effects of orally administrated fermented soy milk (FSM) with low dose of gamma radiation on mammary carcinogenesis induced by exposure to N-methyl-N-nitrosourea (MNU). There was significant immunomodulatory effect of FSM on the reduction of WBC count caused by gamma radiation or MNU. Also FSM showed ameliorative effect on GPx, SOD and CAT enzyme activities and on lipid peroxidation and TNF-α level compared to rats exposed to radiation or MNU. FSM and irradiation markedly elevated GSH content. Combined treatment of FSM and radiation ameliorated MNU effect on cell cycle phases Go/1,S,G2/M. pretreatment with FSM and gamma radiation markedly conclusion, Consumption of FSM synergizes with exposure to low doses of gamma radiation which was found to have a protective and ameliorative effect from carcinogenesis and oxidative stress induced by MNU.

Keywords: Fermented soy, N-methyl-N-nitrosourea, mammary gland, cell cycle, TNF- α, gamma radiation, antioxidant state.

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer death in female worldwide. The significance of nutrition in protecting living organisms from the toxic effects of environmental carcinogens has gained increasing attention due to less toxicity and high efficacy against various diseases. The intake of soy and soy-based products is associated with a lower risk of several types of cancers, including breast cancer. There are many functional ingredients contained in soy foods such as soy protein, isoflavones, saponins, phyticacid, phytosterol, and phenolic acid. The chemo preventive effects of soybean and soy containing food products may be related to genistein, daidzein and glycitein (Khan, 2012).

Soybean fermentation by a system of Lactobacillus and yeast consists of a mixture of soybean extracts and the secondary metabolites of these microorganisms. In addition fermentation increased the bioactive isoflavone aglycone than its unfermented counterpart. It has been used in clinical trials to prevent cancer and cardiovascular disease progression due to its antioxidant activity (Wang et al., 2006), antimutagenic effect also for the reduction of chemotherapy side effects (Chin et al., 2012).

The lactic acid bacteria have cancer chemopreventive properties and act through diverse mechanisms, including

*Corresponding author E-mail: sawsansonbaty@yahoo.com

alteration of the intestinal microflora, enhancement of the host's immune response, and antioxidative and anti proliferative activities (Kim et al., 2007). Some reports also claim that soymilk fermented with probiotic bacteria has some advantages: a reduced content of oligosaccharides, enhanced antioxidant activities, and improved flavor and sensory characteristics (Wang et al., 2003 and Wang et al., 2006). There is evidence suggesting that combining several probiotic bacteria will achieve stronger effects than single-strain probiotics (Chun et al., 2008).

Fermentation of Soy products using different types of microorganisms changes chemical components of soy and increase the soluble nitrogen compounds such as riboflavin, niacin, pantothenic acid, biotin, folic acid and nicotinic acid (Yamauchi et al., 1975; Anderson and Wolf, 1995 and Wang and Wixon, 1999).

Human have always been exposed to various natural sources of ionizing radiation emitted by the isotopes present in the earth's crust, air, water and biosphere, and also originating from the outer space. In some parts of the globe the level of this natural background radiation is significantly higher than the world average with no adverse health effects. Today, people can be additionally exposed to "man-made" radiation delivered at high doses (e.g., during radiotherapy and radiation accidents as well as after detonations of nuclear weapons) or low doses (e.g., during production and distribution of radioactive materials and use of radiation sources for industrial and medical purposes). The low-level environmental and occupational exposures are much more common and distributed over much larger populations than the high-level exposures. The primary aim of this study was to evaluate the effect of fermented soy milk and low dose of gamma radiation on prevention and treatment of induced breast cancer (Nowosielska et al., 2010). This work aim to investigate the protective role of FMS in reducing tumor incidence and progress of N-methyl-N-nitrosourea (MNU) which induced mammary carcinogenesis, accompanied with low dose of gamma radiation.

MATERIAL AND METHOD

Animals

Rats used in this study were Virgin female Sprague-Dawley at 42 days of age, with body weight of 130-150g. Rats were purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt). Animals were housed under standard conditions of light and temperature and allowed free access to standard pellet diet and tap water. Animals were randomly divided into eight groups (n=8).

Fermented soy milk (FSM)

Soy milk was purchased from Soy factor, food technology institute Agricultural research center, Giza, Egypt. The microorganisms used in the fermenting process included: Lactobacillus acidophilus, Lactobacillus bulgaricus, Streptococcus lactis, Bifidobacteria. Fermented soy milk was prepared according to Chang et al.,(2002). FSM was diluted with distilled water to 2%and administrated orally at dose equivalent to 0.2 ml/kg body wt daily.

Four groups of animals were injected with N-methyl-N-nitrosurea (MNU) (Sigma–Aldrich, Diesenhofen, Germany). The MNU was injected intraperitoneally (I/P) twice (50 mg/kg/body weight each), between postnatal days 10 and 30.

Gamma radiation

Irradiation of rats was carried out using a Canadian Gamma cell-40(137 Cs) at the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt. Rats whole body were exposed to gamma rays and received a dose rate of 0.461Gy/minute, calculated according to the Dosimeter department in the NCRRT.

Experimental design and sample collection

Female rats were divided into 8 groups at the beginning of the experiment: group (1): served as negative control and orally received saline, group (2): Rats were injected with MNU, group (3): Animals were exposed to whole body gamma radiation (0.5 Gy), group (4): Rats were given FSM orally via gastric tube (20ml/kg), group(5): rats were given FSM and injected with MNU, group (6): Rats were received FSM (20ml/kg) and exposed to (0.5 Gy) gamma radiation, group(7):Rats were injected with MNU and exposed to (0.5 Gy) gamma radiation, group (8): Rats were given FSM, injected with MNU and exposed to gamma radiation (0.5Gy). At the end of the experiment (13 weeks) animals were anesthetized and sacrificed, Heparinized blood samples were collected from the heart. And mammary glands tissues were dissected.

Evaluation of tumor necrosis factor- alpha (TNF-α)

TNF-alpha concentration in rat mammary gland was measured using the "Assay Max Rat TNF-alpha ELISA kit of murine monoclonal antibody". (ASSAYPRO, 41 Triad South Drive St. Charles, MO 63394, USA).

Evaluation of apoptosis and cell cycle analysis by flow cytometry flow cytometric analysis was performed for cell cycle analysis and evaluation of apoptosis. Mammary glands were cut into small pieces and fixed in 70% ethanol in phosphate buffer saline (PBS) for 1 h on ice, incubated with 50 μg/ml RNase A at 37ºC overnight, stained with 50 μg/ml propidium iodide and subjected to flow cytometric analysis using FACS Calibur. Cells were then analyzed for green (FITC, indicating DNA fragmentation detection) and (PI, allowing DNA quantification) red fluorescence by flow cytometry using a Becton Dickinson® FAC Star Plus flow cytometer.

Table I. The effect of fermented soy milk and and/ or -irradiation treatment on glutathione peroxidase, superoxide dismutase, catalase activities in the blood of tumor bearing rats.

Groups	GPx (mU/mL)	SOD (U/ml)	Catalase (U/L)
Control	6.4 ±0.78[bcd]	24.3 ± 2.9[b]	644 ± 77.3[c]
FSM	2.3 ±0.2[abc]	22.7 ± 2.7[c]	595± 71.2[bc]
Radiation(Rad)	2.1 ±0.2[abc]	21.1 ± 2.5[c]	507 ± 60.4[ab]
MNU	9.9 ± 0.8[acd]	19.5± 2.3[ac]	732 ± 87.4[cd]
Rad+FSM	5 ± 0.6[abcd]	32 ± 3.8[abd]	477 ± 56.9[abd]
MNU+FSM	4.7 ±0.5[abcd]	20.6± 2.5[c]	461 ± 55.1[abd]
MNU+Rad+FSM	8.1 ± 0.16[abcd]	27.9 ± 3.4[bd]	311 ± 37.2[abcd]
FSM+MNU+FSM	2.52 ±0.3[abc]	25.7 ± 3.1[b]	520 ± 74.7[ab]
FSM+ Rad +MNU +FSM	1.4 ± 0.16[abcd]	28.8 ±3.46[abd]	650 ± 77.7[c]

[a] significant compared to control, [b] significant compared to MNU (breast tumor group), [c] significant compare to fermented soy.

Apoptotic cells were identified in a DNA histogram as a sub-G1 hypodiploid population (8).

Antioxidant parameters

Lipid peroxides content (MDA) was determined according to the method of Yoshioka et al. (1979) (7) using 1,1,3,3-tetraethoxypropane as a standard. GSH content was determined according to the method of Beutler et al. (1963) .Glutathione peroxidase determined according to the method of Paglia and Valentine (1967). Catalase activity was estimated according to the method of Sinha (1972) (10). Total protein was determined according to the method of Lowry et al (1970).

Pathological study

Rats mammary gland tissues were fixed in 10% neutral formalin buffer, and then embedded in paraffin wax. Specimens were dehydrated through graded alcohol, cleared in xylene and embedded in paraffin. Sections of 5µm-thickness were cut and stained with Heamatoxylin and eosin (H&E) according to Bancroft and Gamble (2008).

Statistical analysis

Experimental data were analyzed using one way analysis of variance (ANOVA) using SPSS (statistical package for social sciences, 1999; ver.10.0), and the significance among the samples was compared at $P \leq 0.05$. Results were represented as mean ±SD (n =8).

RESULTS

In the present study, MNU intoxication induced significant biochemical alterations in the blood, causing a significant decrease in the GSH content and antioxidant enzymes GPx, SOD and CAT activities also a significant increase in lipid peroxidation products compared to that of control were obtained as shown in Table (1). Oral administration of fermented soy milk(FSM) after MNU injection, significantly ameliorated MDA and GSH contents also GPx, SOD and CAT activities compared to that of the MNU treated group.. Whole body irradiation with low dose (0.5 Gy) markedly raised the enzymatic activity (GPx, SOD and CAT) and GSH content. In addition values of MDA were reduced in all irradiated groups when compared to non-irradiated groups.

Whole body irradiation and oral administration of FSM to MNU treated groups significantly elevated TNF-α levels compared to the control, although tumor groups treated with FSM with or without radiation significantly reduced TNF- value compared to that of tumor group. Pretreatment with FSM with gamma irradiation showed marked amelioration of TNF-α level with increase in GSH content accompanied with reduction in MDA content.

Results in table III representing cell cycle analysis of mammary gland tissue clearly shows that, oral intake of FSM with or without irradiation of rats significantly enhanced apoptotic cell ratio. FSM treatment caused significant alterations in cell cycle analysis as it caused cell cycle arrest at Go/1 appeared in increased cell population at Go/1 with significant decrease in cell population at S and G2/M phases compared to control. Tumor group treated with FSM and whole body irradiation showed amelioration in cell percentage of Go/1,S and G2/M phases compared to control and tumor groups.

Fermented soy milk and gamma radiation effect on tumor bearing rats were evaluated on white blood cells (WBC), lymphocytes (Lym), monocytes (Mon) and granulated cell (Gran) count, in order to detect any potential toxic effects. While FSM significantly reduced lym count, radiation and tumor significantly decreased all differentiated WBC measured parameters (Table IV). FSM showed immunmodulatory effect with radiation on MNU treated group.

Histopathological study by light microscope of female rat mammary glands showed several marked changes with

Table II. The effect of fermented soy milk and /or γ-irradiation on tumor necrosis factor (TNF),glutathione reduced (GSH) and malondialdehyde (MDA)levels in the blood of tumor bearing rats.

Groups	TNF-α (pg/mL)	GSH (mg/dl)	MDA (nmol/L)
Control	25.4 ±3.05^{bcd}	5.0 ± 0.6^{b}	6.1±0.72^{bcd}
FSM	78.2 ±9.34^{abc}	5.8 ± 0.71	2.3 ±0.31^{abc}
Radiation(Rad)	47.5 ±5.69^{abcd}	4.9 ± 0.6^{b}	3.9 ± 0.47^{acd}
MNU	56.5 ±6.77^{acd}	6.6 ± 0.8^{ac}	3.4±0.42^{acd}
Rad+FSM	41.7 ±4.97^{abcd}	8.9±1.08^{abcd}	4.4 ±0.54^{abd}
MNU+FSM	40.5 ±4.85^{abcd}	6.5 ± 0.79^{ac}	2.4 ±0.31^{abc}
MNU +Rad+FSM	22.3 ±2.69^{bcd}	10.3±1.24^{abcd}	5.1± 0.60^{abcd}
FSM+MNU+FSM	71.3 ± 8.55^{ab}	8.4±1.00^{abcd}	3.1 ± 0.35^{acd}
FSM+MNU+Rad+FSM	15.9 ±1.91^{abcd}	13.1±1.56^{abcd}	2.0± 0.23^{abc}

*Legends as in table 1

Table IV. The effect of fermented soy milk and or γ-irradiation on cell cycle analysis and percentage of apoptosis in the mammary gland tissue.

Groups	Go/G1%	S%	G2/M%	Apoptosis%
Control	15.2±1.8^{bcd}	37.2±4.4^{bcd}	5.6±0.7^{bcd}	38.2±4.5^{bcd}
FSM	37.8±4.5^{ac}	14.3±1.7^{abc}	2.04±0.3^{abc}	47.9± 5.7^{abc}
Radiation(Rad)	13.7±1.6^{bcd}	2.7±0.3^{acd}	0.38±0.1^{ad}	84.5± 10.1^{acd}
MNU	33.7±4.0^{ac}	3.2±0.4^{acd}	0.59±0.1^{ad}	79.2± 9.5^{acd}
Rad+FSM	4.3±0.5^{abcd}	0.98±0.1^{ac}	0.42±0.04^{ad}	98.4± 11.8^{abcd}
MNU+FSM	72.8±8.7^{abcd}	18±2.2^{abcd}	9.1±1.1^{abcd}	10.4± 1.3^{abcd}
MNU+Rad+FSM	14.7±1.7^{bcd}	7.5±0.1^{abd}	1.35±0.2^{ab}	73.3± 8.8^{acd}
FSM+MNU+FSM	56.9±6.8^{abcd}	8.9±1.1^{abd}	6.24±0.8^{bcd}	26.1± 3.4^{abcd}
FSM+Rad+MNU+FSM	38.5±4.6^{ac}	5.9±0.7^{ad}	1.2±0.1^{ac}	62.3± 7.5^{abcd}

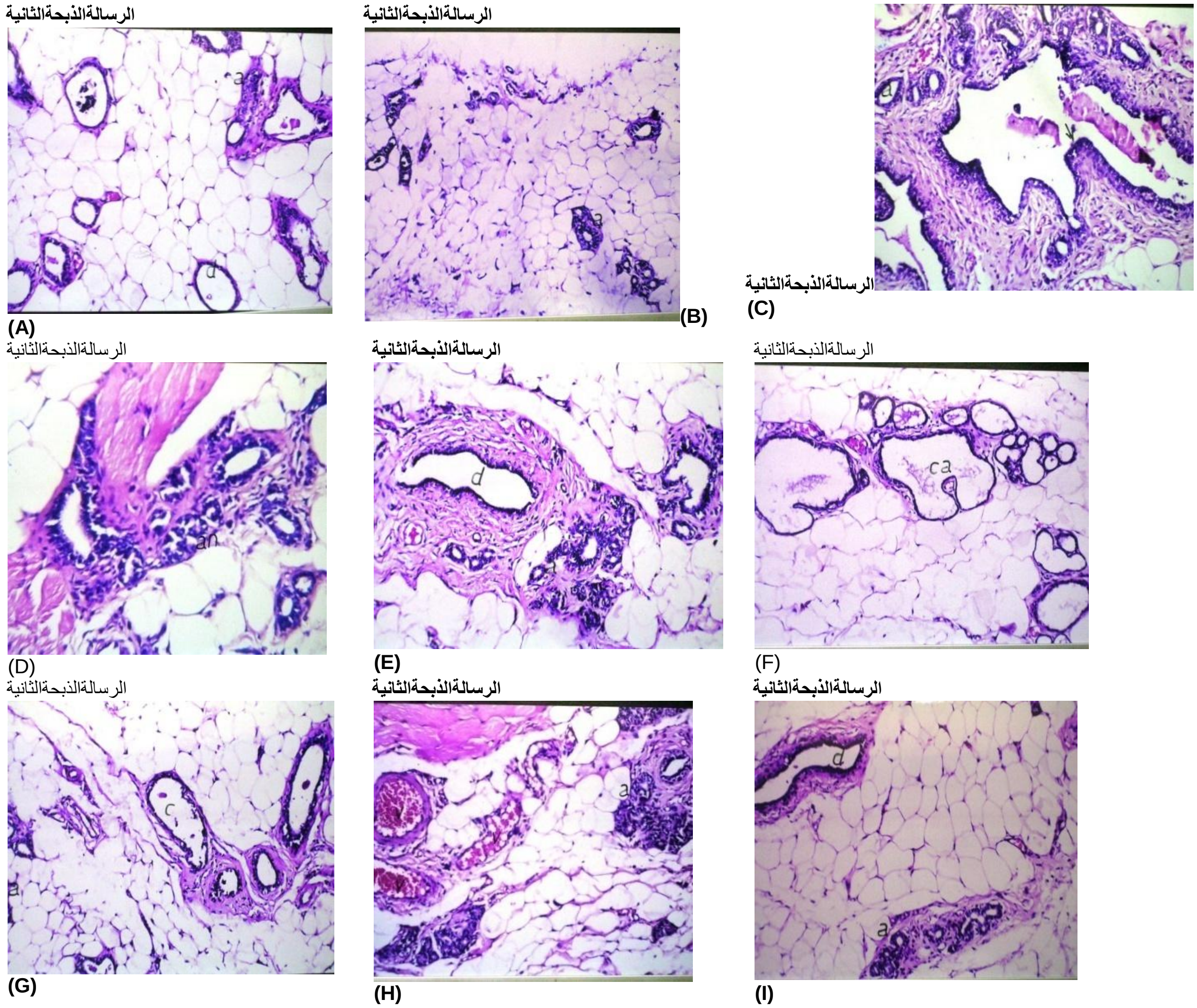
*Legends as in table 1.

Table V. The effect of fermented soy milk and /or γ-irradiation on white blood cells, Lymphocytes, Monocytes and Granulated count/mm^3 of the blood of tumor bearing rats.

Groups	WBC 10M/mm^3	Lymphocyte	Monocyte	Granulated
Control	7.6±0.9^{cd}	5.3±0.7^{cd}	0.8±0.1^{bcd}	1.3±0.14^{cd}
FSM	4.7±0.5^{ab}	3.4±0.4^{ab}	0.4±0.1^{ab}	0.9±0.12^{ab}
Radiation(Rad)	8.3±1.0^{cd}	4.8±0.59^{abcd}	1.0 ±0.1^{abcd}	2.5±0.31^{abcd}
MNU	7.8±0.96^{cd}	5.7±0.66^{cd}	0.6±0.1^{acd}	1.5±0.19^{cd}
Rad+FSM	3.8±0.5^{ab}	2.5±3.1^{abcd}	0.3±0.04^{abcd}	1.0±0.12^{b}
MNU+FSM	7.6±0.96^{cd}	5.3±0.7^{cd}	0.7±0.07^{abcd}	1.7±0.2^{acd}
MNU+Rad+FSM	4.4±0.5^{ab}	2.8±0.4^{abcd}	0.4±0.06^{ab}	1.2±0.14^{c}
FSM+MNU+FSM	9.7±1.1^{abcd}	4.2±0.49^{abcd}	0.8±0.1^{bcd}	4.7±0.54^{abcd}
FSM+Rad+MNU+FSM	5.1±0.6^{ab}	2.5±0.31^{abcd}	0.6±0.07^{acd}	2±0.23^{abcd}

different treatments (fig.1). Mammary gland in the control group was distinguished with lactiferous duct and acini embedded in adipose tissue. Oral administration of FSM revealed normal histological structure, also, mammary gland of irradiated rats with or without FSM administration showed healthy histological structure with no structure alterations. Rats treated with MNU showed anaplastic hyperchromatic lining epithelium with lose of basement membrane (carcinoma). MNU group received FSM showed hyperplasia in lactiferous duct with polyformation and cystic dilation, while treatment with FSM for 15 days before MNU injection markedly ameliorated MNU effect. Female rats treated first with FSM and exposed to gamma radiation then injected with MNU showed normal histological structure

Fig.(1): A: Mammary gland of control rat showing normal histological structure of the lactiferous duct (d) and acini (a) embedded in adipose tissue. B: Mammary gland of rat showing normal intact histological structure of acini (a).C: Mammary gland of irradiated rat showing poly formation in lining epithelium of lactiferous duct (arrow). D: Mammary gland of rat treated with MNU showing anaplastic



hyperchromatic lining epithelium of the acini (an) with lose of basement membrane (carcinoma).E:Mammary gland of irradiated rats+FSM showing normal histological structure of the lactiferous duct (d) and acini (a).F:Mammary gland of rat treated with MNU and FSM showing mild systic dilation of the intact duct(c) and acini.G: Mammary gland of rat treated with MNU and radiation and orally intake of FSM showing intact normal histological structure of the duct (d)and acini (a).H: mammary gland of group orally injected with FSM for 15 days then with MNU showing normal histological structure. I: Section in the mammary gland of rats treated first with FSM and radiation then exposed to MNU with continuous intake of FSM.

DISCUSSION

Functional food enters the concept ofconsidering food not only as a necessity for living but also as a source of mental and physical well-being, contributing to the prevention and reduction of risk factors for several diseases or enhancing certain physiological functions (Lopez-Varela et al., 2002 and Henry, 2010). Fermentation consists of modifying food by microorganisms (bacteria, molds, and yeasts) that grow and reproduce and consume part of the substrate and enrich it with the products of their metabolism. It is an ancient technology that remains one of the most practical methods for preserving foods and enhancing their nutritional qualities (Reddy et al., 1989 and Deshpande et al., 2000). Fermented soymilk, unlike fermented milk or yogurt drinks, contains nolactose or cholesterol and have the health benefits from both soy itself and the fermentation (Lin et al., 2005).

Treatment with N-methyl-N-nitrosourea (MNU) transforms mouse mammary epithelial cells to proneoplastic and neoplastic states in rat. MNU rapidly degrades, so mutagen effect is observed within 24 h (Budan et al., 2008). MNU caused no acute toxicity, but induced both benign and malignant mammary tumors, however, malignant tumors appeared earlier and at a faster rate than the benign tumors (Macejova and Brtko, 2001).

In the present study, ip injection of MNU in female rats caused significant reduction in GPx, SOD, CAT enzymes activities with no significant change in GSH content accompanied with a significant increase in MDA content, this effects were ameliorated with administration of FSM and/or radiation exposure or combined treatment of both FSM and gamma radiation. MNU cause oxidative stress as a result of increasing in free radiacals production result in high significant lipid peroxidation (García-Solís et al, 2005). Antioxidant enzymes are capable of eliminating reactive oxygen species and lipid peroxidation products, thereby protecting cells and tissues from oxidative damage. Superoxide dismutases convert superoxide radicals to molecular oxygen and H2O2, and catalase decomposes H2O2 to molecular oxygen and water. Antioxidant enzyme activities as catalase, Cu/Zn–SOD, and GPx were found to be decreased in tumor tissues and significantly elevated with oral administration ofFSM(Chen et al., 2005), this effect could be due to many antioxidants in soy: soy isoflavones, soy protein, and saponins all possess antioxidative abilities. Soy diet showed significant protection of GSH content in the liver of rats treated with CCl4(Khan, 2012). In addition FSM contains 3-hydroxyanthranilic acid (3-HAA) a by-product of soy fermentation which is able to inhibit lipid oxidation in vivo. (Zhou et al., (2012). fermented soy was effective in reducing lipid peroxidation (Lin et al., 2005).

Fermented soy milk also contains many lactobacillus sp. which exerts potent antioxidant activity and free radical scavenging capability decreasing the risk of ROS accumulation through food ingestion also degrade the superoxide anion and hydrogen peroxide (Liu and pan 2010). Lactobacillus may used to combat oxidative stress, possess several anti-oxidative mechanisms: catalase, glutathione-system-related compounds, and Mn-SOD, attenuation of proliferation caused by reactive oxygen species (Ramesh, et al., 2012) and reduced oxidative stress parameters as NO (Fernanda et al., (2012)

Absorption of low doses of ionizing radiation may stimulate cellular detoxification and repair mechanisms leading to reduction of the DNA damage even below the spontaneous level and decreasing the probability of neoplastic transformation (Redpath and Elmore 2007, Feinendegen et al. 2008). Such exposures may also enhance immune reactions of the organism and attenuate harmful effects of higher doses of radiation (Safwat 2000b, Safwatet al. 2003) Exposure to low dose irradiation (0.5 Gy) significantly delayed the tumor growth, enhanced GSH content in the spleen within 24 hrs post-irradiation (Kojima, 2006).

The inhibition of cytokine production or function serves as a key mechanism in the control of inflammation (Shapira, et al. 1996). In this study we examined the TNFα level in the plasma and found that MNU treatment , exposure to gamma radiation and FMS induce TNFα expression which was markedly ameliorated in tumor group treated with FMS with exposure to low dose of gamma radiation.

elevated TNFα level caused by MNU, Vegh and Salamanca, (2007)found that MNU was ameliorated by soymilk and fermented soymilk which reduce the production of TNF-α, IL-6, IL-1 and PGE2 in the lipid peroxidation induced cells and that may referred to genistein suppressing effect on nitric oxide and COX-2 gene expression(Murakami et al.2003).

In the present study the immunomodulatory activity of FSM against MNU inhibitory effect was clearly observed in total WBCs, Monocytes and granulated cells. Soy ferments probiotic delivery, containing both the bacteria and their potentially bioactive metabolites has the ability to modulate certain aspects of the innate immune system through blood differently depending on the cellular differentiation stage (Budan et al., 2008 Masotti et al. 2011and Vaˇsková et al. 2011).The components of soymilk such as isoflavones, soy protein and saponinable to elicit immune response(Kobayashi et al 2005). However, fermentation change components of these compounds, fermentation with lactic acid bacteria and/or bifidobacterium increased aglycone with a decrease of glucoside isoflavones content in soymilk. The fermented soymilk also contained components of lactic acid bacteria or bifidobacterium cells and peptides formed during the fermentation, which have been reported to affect the production of cytokines. Therefore, the variation in the immune response observed with fermented soymilk may be attributed to its composition. Leblanc et al., (2004) reported that fermented soy by lactic acid bacteria significantly increase the phagocytic index. They also found that, heat treated fermented products were able to stimulate the innate immune response. Exposure to low dose of gamma radiation (0.5 Gy) selectively decrease the percentage of B cells in blood lymphocytes concomitant with an increase in the helper T cell population(Kojima ,2006).

Our data showed that cell cycle analysis study in the female rat mammary tissue via flow cytometry showed that, MNU caused increase in apoptotic cell count. Also disturbance in cell cycle observed in all phases with accumulation of cell count at G1, this disturbance was significantly ameliorated by FSM treatment and pretreatment while combined treatment of FSM and gamma radiation exerted marked effect on decreasing MNU effect on cell cycle analysis and apoptotic cell count compared to the control.

MNU has an impact on the expression of regulatory genes triggering apoptosis in 12 h and directly development toxicity followed by accumulation of mutations either in somatic cells or blood cells (Budan et al., 2008 and Vaˇsková et al. 2011). The proposed antiproliferative effects of FSM reflect the primary protective action on damaged cells. Induction of apoptosis may be considered in case of failure of reparative mechanisms lead to cell death and is alsoimportant for protection of the entire organism. Low dose of radiation induce inhibitory effect on tumor cell, it is significantly delayed the tumor growth in Ehrlich solid tumor bearing mice (Kojima,2006).

In the present study, the histological observations indicate that MNU treatment caused carcinoma, anaplastic hyperchromatic lining epithelium. Oral administration of FSM before and after MNU administration had ameliorative effects against carcinogenesis effect induced by MNU. It has been shown that FSM accompanied with low dose of gamma radiation has great efficiency as, anti-inflammatory and antitumor treatment against MNU carcinogenesis. The ameliorative effects of FSM upon the structural alterations could be explained by the role of FSM in regulating vital cellular functions, including cell proliferation and differentiation and its potent antioxidant activity and free radical scavenging capability.

This study demonstrates the role in vivo of FSM in the inhibition of mammary tumor induced by MNU which were mammary tumors in which inhibitory effects were observed in histopathological result as well as with significant tumor regression, beside it exerted suppression effects on TNF alpha and MDA levels. Fermented soymilk has been found to possess antioxidant and antimutagenic activity. Therefore, this fermented soymilk could potentially be used as a dietary therapy to reduce the risks of breast cancer, and as treatment accompanied with low doses of gamma irradiation.

REFERENCES

Chen YC, Sugiyama Y, Abe N, Kuruto-Niwa R, Nozawa R, Hirota A. (2005): DPPH radical-scavenging compounds from Dou-chi, a soybean fermented food. Biosci. Biotechnol Biochem;69:999-1006.

Chen, Y. C., Shen, S. C., Lee, W. R., Hou, W. C., Yang, L. L. and Lee, T. J. F. 2001.Inhibition of nitric oxide synthase inhibitors and lipopolysaccharide inducible NOS and cyclooxygenase-2 gene expression by rutin, quercetin, and quercetinpenta acetate in RAW 264.7 macrophages. J. Cell. Biochem. 82: 537-548.

Chin, Y.; Tsui, K.; Chen, M.; Wang, C.; Yang, C. and Lin, Y.(2012): Bactericidal Activity of Soymilk Fermentation Brothby In Vitro and Animal Models. J Med Food 15 (6) ; 520–526.

Ching-Yi Lin, Zheng-Yu Tsai, I-Chi Cheng, Shyh-Hsiang Lin(2005): Effects of fermented soy milk on the liver lipids underoxidative stress. World J Gastroenterol, 11(46):7355-7358.

Chun J, Woo JJ, Kim JS, Lim J, Park CS, Kwon DY, Choi I, Kim JH (2008): Hydrolysis of isoflavone glucosides in soymilk fermented with single or mixed cultures of Lactobacillus paraplantarum KM, Weissella sp. 33, and Enterococcus faecium 35 isolated from humans. J Microbiol Biotechnol;18:573–578.

De Nardin, E. 2001. The role of inflammatory and immunological mediators in periodontitis and cardiovascular disease. Ann. Periodontol. 6: 30-40.

Deshpande, S. S.; Salunkhe, D. K.; Oyewole, O. B.; Azam-Ali, S.; Battcock, M.; Bressani, R. Fermented Grain Legumes, Seeds and Nuts. A Global PerspectiVe; Food and Agriculture Organization of the United Nations: Rome, Italy, 2000.

Ewa M. Nowosielska, AnetaCheda, Jolanta Wrembel-Wargocka, Marek K. Janiak

Field CJ, Johnson IR, Schley PD (2002): Nutrients and their role in host resistance to infection. J Leukoc Biol;71:16–32.

Gibson RS (2007): The role of diet- and host-related factors in nutrient bioavailability and thus in nutrient-based dietary requirement estimates. Food Nutr Bull; 28(1Suppl International):S77–S100.

Henry CJ (2010): Functional foods. Eur J ClinNutr; 64:657–659.

Immunological mechanism of the low dose radiation induced suppression of cancer metastases in a mouse model. Dose-Response,8:209–226, 2010

JankaVaˇskovaa,, Monika Kassayovab, LadislavVaˇskoa (2011): Potential role of melatonin in DNA damage caused by nitrosourea-inducedmammary carcinogenesis ActaHistochemica 113 (2011) 423–427

Kim JE, Hur HJ, Lee KW, Lee HJ (2007): Anti-inflammatory effects of recombinant arginine deiminase originating from Lactococcuslactis ssp. lactis ATCC 7962. J Microbiol Biotechnol;17: 1491–1497.

Kin, S., Sasaki, T., Gu, K., Nakayama, K., Tamura, K., Nakai, I. and Oka, T. 1995. The cytoprotective role of nitric oxide in ischemia-reperfusion injury in the rat kidney.Transplant. Proc. 27: 754-756.

Kobayashi, H., Yoshida, R., Kanada, Y., Fukuda, Y., Yagyu, T., Inagaki, K., Kondo, T., Kurita, N., Suzuki, M., Kanayama, N. and Terao, T. 2005.Suppression of lipopolysaccharide-induced cytokine production of gingival fibroblasts by a soybean, Kunitz trypsin inhibitor. J. Periodont. Res. 40: 461-468.

Kojima S. (2006) : Induction of glutathione and activation of immune functions by low-dose, whole-body irradiation with gamma-rays].YakugakuZasshi. 126(10):849-57.

Leblanc, J.G.; Garro, M.S.; Savoy de Giori, G. and Font de Valdez,G. (2004): A Novel Functional Soy-based Food Fermented by Lactic Acid Bacteria: Effect of Heat Treatment.JOURNAL OF FOOD SCIENCE—Vol. 69,246-250.

Lin, C.; Tsai, Z.; Cheng, I. and Lin, S. (2005): Effects of fermented soy milk on the liver lipids underoxidative stress. World J Gastroenterol;11(46):7355-7358.

Lopez-Varela S, Gonzalez-Cross M, Marcos A (2002): Functional foods and the immune system: a review. Eur J Clin Nutr;56 (Suppl3):S29–S33.

Marnett, L. J. 1992. Aspirin and the potential role of prostaglandins in colon cancer. Cancer Res. 52: 5575-5589.

Masotti , A.I. Buckley N.. Champagne C.P , J. Green-Johnson (2011). Immunomodulatory bioactivity of soy and milk ferments on monocyte and macrophage models Food Research International 44 2475–2481

Murakami, A., Matsumoto, K., Koshimizu, K. and Ohigashi, H. 2003. Effects of selected food factors with chemopreventive properties on combined lipopolysaccharide- and interferon-γ-induced IB degradation in RAW 264.7 macrophages. Cancer Lett. 195: 17-25.

Reddy, N. R., Salunkhe, D. K. Fermentation. In Handbook of World Food Legumes: Nutritional Chemistry, Processing Technology and Utilization; CRC Press, Inc.: Boca Ratón, FL, 1989;Vol. III.

Sarsilmaz M, Songur A, Ozyurt H, et al. (2003): Potential role of dietary omega-3 essential fatty acids on some oxidant/antioxidant parameters in rats' corpus striatum. Prostaglandins Leukot Essent Fatty Acids.;69:253–9.

Shapira, L., Soskolne, W. A., Houri, Y., Barak, V., Halabi, A. and Stabholz, A. 1996. Protection against endotoxic shock and lipopolysaccharide-induced local inflammation by tetracycline: Correlation with inhibition of cytokine secretion. Infect. Immun. 64: 825-828.

Unemori, E. N., Ehsani, N., Wang, M., Lee, S., McGuire, J. and Amento, E. P. 1994. Interleukin-1 and transforming growth factor-α: synergistic stimulation of metalloproteinases, PGE2, and proliferation in human fibroblasts. Exp. Cell Res. 210: 166-171.

Wang YC, Yu RC, Chou CC (2006): Antioxidative activities of soymilk fermented with lactic acid bacteria and bifidobacteria. Food Microbiol;23:128–135.

Wang YC, Yu RC, Yang HY, Chou CC(2003): Sugar and acid contents in soymilk fermented with lactic acid bacteria alone or simultaneously with bifidobacteria. Food Microbiol; 20: 333–338.

Wen- Huei Chang , Jun – Jen Liu , Ching- Hsein Chen , Tien – Shang Huang , and Fung – Jou Lu 2002 national and cancer 43: 214 - 226

Yen, G. C., and Lai, H. H. 2003. Inhibition of reactive nitrogen species effects in vitro and in vivo by isoflavones and soy-based food extracts. J. Agric. Food Chem. 51: 7892-7900.

Zhou, S.;Zhang, R. Xu, Y. and Bi, T. (2012): Antioxidant and Immunity Activities of Fufang Kushen Injection Liquid. Molecules 2012, 17, 6481-6490.