

IMMUNO-EFFECT OF RAW, GAMMA IRRADIATED, MICROWAVE TREATED AND FERMENTED WHEAT GERMS IN EXPERIMENTAL RATS.

Youssef, M. K. E.*; F. M. El-Tellawy **; Naglaa T. El-Melegy* and Moshera M. H. Mohamed . ******

*** Food Science & Technology Dept., Fac. of Agric., Assiut University. Correspondence should be addressed,**

e-mail: Kyoussef7@yahoo.com, Fax: +2088 2331384

**** Home Economic Dept., Fac. of Specific Education, Ain Shams Univ.**

***** Medical Biochemistry Dept., Fac. of Medicine, Assiut University.**

****** Home Economic Dept., Fac. of Specific Education, Assiut University.**

ABSTRACT

In the present study on effect of feeding raw wheat germ, microwave treated wheat germ, gamma irradiated wheat germ and fermented wheat germ on Interferon gamma (IFN- γ) and Interleukin 10 (IL-10) in serum of rats were investigated. The data indicated that treated and untreated wheat germ had significant increase in (IFN- γ) level in the serum of rats. Fermented wheat germ recorded the highest significant increase in this concept in comparison to control group. Wheat germ supplementation indicated significant changes of (IL-10) level in serum of rats in comparison to control group. Fermented wheat germ recorded the least significant decrease in (IL-10) level in serum of rats in comparison to control group. Treated and untreated wheat germ specially fermented wheat germ raised the immune system of experimental rats. On the other hand, the present study included the vitamins content (vitamins A, E, and C) of raw, microwave treated, gamma irradiated, and fermented wheat germ clarifying the role of each vitamin in raising the immune system.

Keywords: Raw wheat germ, gamma irradiated wheat germ, microwave treated wheat germ, fermented wheat germ, vit. A, vit. E, vit. C, (IFN), (IL-10).

INTRODUCTION

Two types of immunity protect the body: innate and adaptive. Innate immunity is present at birth and provides the first barrier against microorganisms. Adaptive immunity is the second barrier to infection. It is acquired later in life, such as after an immunization or successfully fighting of an infection. The immune system is the body's primary defensive survival mechanism. It can determine what is "self", which needs to be protected and what is "non-self", which needs to be destroyed. A properly functioning immune system allows us to live a life virtually free of illness and disease (Bowers, 1997). Nutrition plays a key role in maintaining optimal immune function. Recent research had shown that might, if not all, of the body's defenses could be hindered by malnutrition. Cell mediated immunity; antibody production, inflammatory response and secretory and mucosal immunity are some of these functions. Nutritional excesses can also alter the immune response (Wardlaw, 1999).

Wheat germ is rich in polyunsaturated fats (~12%), mainly oleic, linoleic, and α -linolenic acids, and vitamins, especially vitamin E, A and B. Its

protein content is ~ 28 % and hence wheat germ is a source of essential amino acids with improved nutritional potential if compared to other cereal products. High lipase and lipoxygenase activities also characterize wheat germ. (Sjovall *et al.*, 2000). Whole grain, such as wheat germ are rich sources of dietary fiber, vitamins, minerals and phytochemicals including phenolics, carotenoids, vitamin E, lignans, β -glycan, inulin, resistant starch sterol and phytates (Liu, 2007). Plant based foods contain significant amount of bioactive phytochemicals, which when consumed together may have a synergistic affect that goes beyond the basic individual function of each single component in combating diseases (Liu, 2007). Wheat germ is therefore sensitive to oxidation that may cause destruction of essential fatty acids and vitamins . Oxidation could be prevented, and shelf life prolonged, by inactivating the enzymes under heat treatment or by removing the oil fraction from wheat germ by extraction, or combined techniques. (Sjovall *et al.*, 2000).

There are two types of antioxidants: antioxidant enzymes and nonenzymatic, antioxidant including vitamins E and C, caroteins and phytochemicals. They work by intercepting and stabilizing the reactive oxygen species. Adequate vitamin and mineral intake is nessary for an optimally functioning immune system. Deficiencies and/or excesses of these nutrients can alter the immune response. Understanding the effects of not only nutrient deficiencies but in light of the widespread use of vitamin and mineral supplements excesses as well is crucial (Diplock, 1995).

Wheat germ is the nutritionally richest part of the seed. It is one of the few plant parts in nature in which the entire vitamin B-complex is found. It also contains fiber and vitamin E (Wood, 1999). In addition, wheat germ oil , rich in vitamin A , has a full balance of mixed tocopherols (α , β - and γ -tocopherols) that makes it much more bio-available than other synthetic sources of vitamin E. Furthermore, α -tocopherol is free radical scavengers that give wheat germ oil its potent antioxidant qualities . (Jayaraj *et al.*, 2001). Besides, Wheat germ oil is one of the most concentrated natural sources of vitamin E. According to the (USDA, 2006), 1,100 mg of non-GMO wheat germ oil (the amount that is in each vegetarian soft gel cap of Wheat Germ Oil E (TM) contains over 164mg of vitamin E in the form of alpha-tocopherol (USDA, 2006).

Food irradiation and microwave may play an important role in meeting this challenge. Irradiation technology can be used to process many types of foods, by exposing them to appropriate levels of radiation doses. The United States Department of Agriculture (USDA) and the United States Food and Drug Administration (FDA) in 1983 had approved the use of gamma irradiation on a variety of food products for a number of different purposes like the elimination of microbial contamination. It is the most cost-effective technology and is preferred by many processors because the good penetration enables administering treatment to entire industrial pallets or totes, greatly reducing the need for material handling. Food irradiation is recognized as a safe and effective process for a range of specific application, among them the disinfestations of many food items including cereal grains, legumes, fresh and dried fruits, nuts, dried vegetables (W.H.O), 1994). The treatment by gamma rays is regarded as a versatile and effective alternative

of chemical fumigants to combat pests combat insect pests. A irradiated food is wholesome and nutritionally adequate. The FAO/WHO/IAEA Joint Expert Committee on Food Irradiated has unconditionally cleared food irradiated up to 10 kGy as safe for human consumption. Therefore, radiation processing could offer promise to minimize post-harvest losses provided the nutritional quality is not impaired by radiation processing (Diehl, 1995). Moreover, radiation treatment had been suggested to inactivate or reduce antinutritional factors in cereals (Farag, 1998).

In the present study, some processing methods were used to improve the stability of wheat germ. Such methods included microwave, gamma irradiation and fermentation aiming to destroy the antinutritional factors present in the wheat germ. In addition to increase nutritional value of wheat germ to raise the protection percentage from autoimmune diseases and raise the immune system as well.

MATERIALS AND METHODS

Materials:

Source of samples:

2 wheat germ varieties namely: Giza 60 and Saha 40 were mixed in the Asdekaa mill and the resultant wheat germ was used in the present study. 20 Kg sample of wheat germ was procured from this mill during March 2008.

Treatment of wheat germ sample :

Twenty kilograms sample was divided into four samples. The first 5.0 Kg. sample (raw wheat germ) was used as control. The second 5.0 Kg. sample of raw wheat germ was irradiated at Egyptian Atomic Energy Authority using a ⁶⁰Co gamma source at an average dose of (6 kGy), which is the average dose recommended by the (Codex Alimentarius commission (1990), to reduce microbial load and the number of pathogenic microorganisms for wheat germ. The third 5.0 Kg sample of raw wheat germ was treated by microwave radiation. The treatment using microwave radiation (MS143SCE, Samsung Electronics, Korea) was performed by continuous supply of wheat germ in the pasteurization chamber at 200 W/Kg of wheat germ for 3 min.

The wheat germ was subjected to microwave heating for 3.0 min. at 200 W using a household microwave oven. Sample was placed in a pyrex Petri dish (8.0) cm diameter by 1.0 cm depth; each Petri dish contained approx. 25g) and heated for the required period. After that the wheat germ samples were allowed to cool at room temperature (Yousif, 2004). On the other hand, the fourth 5.0 Kg. sample of wheat germ was fermented as described by (Hidvègi *et al.*, 1999).

Methods:

(1) Chemical methods:

Determination of vitamin contents:

Vitamin C was determined according to (Bajaj and Kaur, 1981). Besides, vitamins E. and A. were determined according to the methods described by the (Principal Central Lab. of Cairo University, 2008).

Experimental animals

Sixty three adult male white albino rats, weighing between 135 and 140 grams were obtained from the animal house of the Faculty of Medicine, Assiut University. The animals were kept in wire cages under the normal laboratory conditions. Rats were randomized into (9) groups of (7) animals each. The first group was fed on basal diet (control), the second group was fed on basal diet with 5% of raw wheat germ, the third group was fed on basal diet with 10% of raw wheat germ, the fourth group was fed on basal diet with 5% of microwave treated wheat germ, the fifth group was fed on basal diet with 10% of microwave treated wheat germ, the sixth group was fed on basal diet with 5% of gamma irradiated wheat germ, the seventh group was fed on basal diet with 10% of gamma irradiated wheat germ, the eighth group was fed on basal diet with 5% of fermented wheat germ, and the ninth group fed on basal diet with 10% of fermented wheat germ. This modified powdered diet was fed to rats throughout the experimental period of two months to the experiment for acclimatization, and tap water was provided. Rats were housed individually in wire cages under the normal laboratory conditions and was fed on the basal diet for a week as adaptation period. The animals were cared for in compliance with the principles and guidance of ethical committee for animal care and use. The basal diet used consisted of corn oil, salt mixture, vitamin mixture and cornstarch as described by (Mohamed 2005). Each rat was marked on the tail to differentiate between animals. Daily administrations were continued for two months.

Blood samples:

Five ml blood samples were obtained from each rat by eye vein before scarification of rats puncture under complete aseptic conditions. Blood samples were taken into plain tubes, allowed to clot for 2 hours at room temperature before centrifuging for 20 minutes at approximately 1000 x g. Serum samples were stored into aliquots at -20° C till time of assay.

(2) Biochemical methods:

Determination of interferon gamma (IFN- γ) and interleukin-10 (IL-10) in rats

Serum levels of rat IFN- γ were determined by Rat IFN- γ Immunoassay Kit. Catalog Number RIF00 by Quantikine, R & Systems Inc, USA.

Serum levels of rat IL-10 were determined by Mouse/Rat IL-10 Immunoassay Kit. Catalog Number M2200 by Quantikine, R & Systems Inc, USA.

Statistical analysis:

Data were tested for normality and were found to be normally distributed. Accordingly, data are presented as the mean \pm SD. Statistical differences between groups were assessed using paired and unpaired Student t test where appropriate. Repeated measures analysis of variance (ANOVA) was used to compare differences in serial samples. A value of <0.05 was considered statistically significant. Recovery was calculated by dividing the detector response of analyze in the sample to the response of analyze reference material. Correlation analysis between the different studied variables was performed using spearman's rank correlation coefficient. All analyses were performed using Statistical Package for Social Sciences (SPSS) software (version 11.0).

RESULTS AND DISCUSSION

Vitamins contents:

The data given in Table (1) revealed that fermented wheat germ recorded the highest percentages in Vitamin (A) and Vitamin (C) (16555.70 IU/100g) and (125.82 mg/100g); respectively. However microwave treated wheat germ had the highest content of vitamin (E) (1840 IU/100g).While gamma irradiated wheat germ recorded the lowest percentages of Vitamin (A) and Vitamin (C) (7941.80 IU/100g and 87.78 mg/100g) ; respectively. On the other hand, raw wheat germ recorded the lowest percentages of Vitamin (E) (1312 IU/100g).

Table (1):Vitamins content of raw wheat germ, gamma-irradiated wheat germ, microwave treated wheat germ and fermented wheat germ.*

Treatments	Vitamin(A) IU/100g	Vitamin (E) IU/100g	Vitamin (C) mg/100g
Raw wheat germ	10343.23	1312	98.45
Gamma irradiated wheat germ	7941.80	1360	87.78
Microwave treated wheat germ	16311.10	1840	106.17
Fermented wheat germ.	16555.70	1552	125.82

*Mean of three replicates.

Serum levels of interferon gamma (INF-γ) in Albino rats fed on wheat germ with different treatments and basal diet:

Serum levels of IFN-γ in Albino rats fed on wheat germ with different treatments and control are presented in table (2). The data revealed that there was significant increase in serum levels of INF-γ in groups V(basal diet with 10% of microwave wheat germ) (P < 0.01), VI (basal diet with 5% of gamma irradiated wheat germ), VII (basal diet with 10% of gamma irradiated wheat germ), VIII (basal diet with 5% of fermented wheat germ), IX(basal diet with 10% of fermented wheat germ) (P<0.001) for each in comparison to group I basal diet (control).Although level of INF-γ in group II, (basal diet with 5% of raw wheat germ),III (basal diet plus 10% of raw wheat germ),IV(basal diet with 5% of microwave wheat germ) were higher than that the fact that of group I but the difference did not reach the level of significance.

This might be due to wheat germ is rich sources of dietary fiber, vitamins, minerals and phytochemicals including phenolics, carotenoids, vitamin E, lignans, β-glycan,inulin, resistant starch sterol and phytates (Liu, 2007).

Deficiencies, for any reason, affect all components of immunity. Among the causes the deficiency are an inadequate diet, impaired digestion and /or absorption, altered metabolism, a disease state, increased utilization of a nutrient and increased need for a nutrient. Excesses of specific nutrient can also alter immune function. Certain nutrients, including vitamins C and E, affect various parts of immune function when present in excess quantities (Bowers, 2002).The effects of malnutrition on immunity had been extensively studied and were well documented (Scrimshaw,1997). In case of protein energy malnutrition there is increased morbidity and mortality from infections

due to suboptimal immune systems. The increased rate of infection and death in protein energy malnutrition is due to the inability of the immune system to resist and/or effectively fight the infection. Without adequate vitamin E the immune system does not function normally. Phagocytosis as well as cell mediated and humoral immunity are all impaired. Vitamin E supplementation appears to enhance immunity and resistance to infections in animals and quite possibly humans (Meydani,1996).Vitamin E has immune functions beyond its role as an antioxidant, such as regulation the synthesis of eicosanoids on immune cell membranes, keeping the cells from overproducing eicosanoids. When vitamin E is deficient, production of the immunosuppressive PGE₂, is increased, which impairs immune function (Bendich, 1990).Vitamin E supplementation increase differential count (lymphocytes and monocytes).(Abdel Ghafar,2004).On the other hand, vitamin E deficiency induces the decrease differentiation of immature T cells which results in the early decrease of cellular immunity(Moriguch and Muraga, 2000). Vitamin E enhances some measures of immune-cell activity in the elderly (Meydani *et al.*,1990). This effect was more pronounced with 200 IU per day, while under 200 IU per day had not boosted immune function in some reports (De Waart *et al.*, 1997).Beta-carotene and other carotenoids had increased immune cell numbers and activity in animal and human research. An effect that appears to be separate from their role as precursors to Vitamin A. (Chew,1993). Placebo-controlled research had shown positive benefits of beta-carotene supplements in increasing numbers of ,some white blood cells and enhance in cancer- ling Immune functions in healthy people at 25,000-100,000 IU per day(Hughes *et al.*, 1997).In double-blind trials in the elderly, supplementation with 40.000-150,000 IU per day of beta-carotene has increased natural killer (NK) cell activity (Santos et al,1996), but not several other measures of immunity(Santos et al,1997).

Vitamin C stimulates the immune system by both elevating interferon levels (Gerber,*et al.*,1975)and enhancing the activity of certain immune cells (Anderson,1984). Vitamin C improves immune function in the elderly (Delafuente *et al.*,1986)

Vitamin A plays an important role in immune system function and helps mucous membranes, including those in the lungs, resist invasion by microorganisms (Semba,1994). However, most research showed had while vitamin A supplementation helped people to prevent or treat infections in developing countries where deficiencies are common (Glasziou and Mackerras,1993).

Plant lectins, such as wheat germ agglutinin constitute common components of the human diet and target the immune system on a daily basis. The wheat germ-deprived diet induced a state of functional unresponsiveness in lymphocytes from primary and secondary lymphoid organs, as evaluated by in vitro stimulation with T cell mitogen phytohaemoagglutinin and B cell mitogen lypopolysaccharides. The unresponsive state on the immune cells colud be revesed by injection of antigen emulsified in oil with inactivated mycobacteria . Dietary signals can thus interact with the immune system possibly influencing its shaping during ontogenesis.(Chignola *et al.*, 2002) .

Table (2) : Serum levels of interferon gamma(INF-γ)in Albino rats fed on wheat germ with different treatments and basal diet (pg/mL):

Groups(n=7)	Parameters	INF- γ (pg/mL)	Range
		Mean ± S.D.	
Group I (Basal diet) Control		33.43 ± 3.26	30.00 -38.00
GroupII (Basal diet with 5% of RWG)		33.29 ± 3.25 a ^o	29.00 - 38.00
GroupIII(Basal diet with 10% of RWG)		36.71 ± 90 a ^o b ^o	32.00 – 43.00
GroupIV(Basal diet with 5% of MWG)		42.00 ± 4.65 a ^o b ^o c ^o	35.00 – 48.00
GroupV(Basal diet with 10% of MWG)		47.43 ± 5.16 a** b** c* d ^o	40.00 – 55.00
GroupVI (Basal diet with 5% of GWG)		71.14 ± 11.22 a*** b*** c*** d*** e***	55.00 – 85.00
GroupVII(Basal diet with10% of GWG)		79.71 ± 11.93 a*** b*** c*** d*** e*** f ^o	60.00 – 95.00
Group III (Basal diet with 5% of FWG)		94.57 ± 10.39 a*** b*** c*** d*** e*** f*** g**	80.00 – 110.00
GroupIX (Basal diet with 10% of FWG)		108.57 ± 12.82 a*** b*** c*** d*** e*** f*** g*** h**	90.00 – 125.00

RWG = Raw wheat germ.

MWG = Microwave treated wheat germ.

WG =Gamma irradiated wheat germ.

FWG = fermented wheat germ.

* = P < 0.05 ** = P < 0.01 *** = P < 0.001 ^o : non-significant.

a : Group II, III, IV, V, VI, VII, VIII, IX versus group (control).

b : Group III, IV, V, VI, VII, VIII, IX versus group II.

c : Group IV, V, VI, VII, VIII, IX versus group III.

d : Group V, VI, VII, VIII, IX versus group IV.

e : Group VI, VII, VIII, IX versus group V.

f : Group VII, VIII, IX versus group VI.

g : Group VIII, IX versus group VII

h : Group IX versus group VIII.

Results revealed that the mean serum levels of INF-γ in groups V(basal diet with 10% of microwave wheat germ) (P < 0.01), VI (basal diet with 5% of gamma irradiated wheat germ), VII (basal diet with 10% of gamma irradiated wheat germ), VIII (basal diet with 5% of fermented wheat germ),and IX(basal diet with 10% of fermented wheat germ) (P<0.001)recorded significant increase in for each in comparison to group II(basal diet with 5% of raw wheat germ). Meanwhile the mean serum levels of INF-γ in groups V (P < 0.05) VI, VII, VIII, IX (P<0.001) had significant increase for each in comparison to group III (basal diet with 10% of raw wheat germ).

This might be due to wheat germ is therefore sensitive to oxidation that may cause destruction of essential fatty acids and vitamins (Sjovall *et al.*2000). Oxidation could be prevented, and shelf prolonged, by inactivating the enzymes under heat treatment or by removing the oil fraction from wheat germ by extraction, or combined techniques (Sjovall *et al.*,2000). Extrusion cooking (Ekstrand *et al.*1993) and microwave heating (Kermasha *et al.* 1993) had been reported to be rapid and effective methods for inactivating oxidative enzymes. However, over the last years a trend towards an increased consumption of minimally processed plant food, wheat germ included ,is observed resulting in a higher intake of antinutritional compounds such as lectins.

On the other hand mean serum levels of INF-γ in groups VI, VII, VIII, IX (P<0.001) recorded significant increase for each in comparison to group IV (basal diet with 5% of microwave wheat germ).

This might be due to that the nutritive quality or digestibility of plant protein is affected by the presence of antinutritional factors such as proteinase inhibitors, especially trypsin and chymotrypsin inhibitors (Abu-Tarboush and Ahamed, 1996). Several conventional processing methods, such as soaking and fermentation had been used to inactivate these undesirable components from plant seeds. (Hassan and El Tinay, 1995).

The above-mentioned treatments generally reduced antinutritional factors, but the effect varied with plant cultivars and treatments. In many cases, the use of only one method might not affect the desired removal of antinutritional factors thus combination of two or more methods were required. Moreover, destruction of some nutrients and loss of some water-soluble nutrients may occur with heat and soaking treatments (Abu-Tarboush, 1998).

The results revealed that mean serum levels of IFN- γ in groups VIII, IX ($P < 0.001$) recorded significant increase for each in comparison to group VI. Moreover, mean serum levels of IFN- γ in groups VIII ($P < 0.01$), IX ($P < 0.001$) for each in comparison to group VII were noted.

This might be due to that during the fermentation of wheat germ with yeast quinines were released by the glycidase enzyme of the yeast fungus. The original perception of (Szent-Györgyi, 1982) was that by mean of the biological activity of these released quinines, the fermented wheat germ might possess immunostimulatory effect.

Beta-glucan is a fiber-type polysaccharide (complex sugar) derived from the cell wall of baker's yeast activates white blood cells. (Wakshull *et al.*, 1999). Beta-1,3- glucan is very effective at activating white blood cells known as macrophages and neutrophils. A beta- glucan- activated macrophages or neutrophils can recognize and kill tumor cells, remove cellular debris resulting from oxidative damage speed up recovery of damaged tissue, and further activate other components of the immune system (Ross *et al.*, (1999).

The results agree with (Hidvégi *et al.*, 1999) findings who reported that evidence of the immunomodulatory effects of Avemar was first obtained in a study on the effect of the compound on immune function in mice. Avemar significantly increased the blastic transformation of peripheral blood T lymphocytes stimulated by concanavalin A. From a therapeutic point of view, the immunomodulatory and immunorestoring effects of Avemar may be exploited in various clinical manifestations of impaired immune response. showed that MSC (AVEMAR) pre-treatment of mice increased the plastic transformation on peripheral T lymphocytes. As the stimulatory effect on lymphocytes was exerted by *in vivo* treatment, the relatively moderate augmentation of lymphoblast transformation might be considered as important.

Avemar is the product of industrial fermentation of wheat germ. Since its invention, a series of *in vitro* and *in vivo* studies and clinical trials had been carried out to determine whether Avemar could help cancer patients struggling with both the effects of their disease and the side effects of standard anticancer therapy (SAT). Subsequently, evidence of the efficacy of

the fermented wheat germ extract in some autoimmune diseases had also been found.(Boros *et al.*,2005)

Oral intake of Avemar could ameliorate the clinical manifestations of experimental systemic lupus erythematosus SLE by affecting the Th1/Th2 network inhibiting the Th2 response. (Sukkar and Rossi,2004). Avemar is a fermented wheat germ extract introduced as a nontoxic dietary supplement with anticarcinogenic effect.Furthermore , mean serum levels of INF- γ in groups IX(basal diet with 10% of fermented wheat germ) recorded significant increase each in comparison to for each group VIII (basal diet with 5% of fermented wheat germ).

This might be due to the higher percentage of fermented wheat germ given in diets of those groups.

Serum levels of interleukin-10 (IL-10) in Albino rats fed on wheat germ with different treatments and basal diet :

A regard IL-10 there was significant increase in levels of IL-10 in group II, III, IV, V in comparison to group I . Although level of IL-10 in groups VI, VII, VIII, IX showed lower levels in comparison to group I but the difference did not reach the level of significance.

There was significant decrease in level of IL-10 in groups VI (P <0.05), VII (P<0.01), VIII (P < 0.001), IX (P < 0.001) in comparison to group II. However, a significant increase in level of IL-10 was observed in group V in comparison to group II (P <0.001).

Extracts of cereal grains, including rice and wheat, induced marked IL-10 production from PBMCs. Intracellular cytokine staining and cell-depletion experiments showed that CD14+ monocytes produced IL-10. Importantly, when PBMCs were stimulated with concanavalin A, cereal grains concentration-dependently inhibited their production of IL-5, IL-13, and IFN- γ ; neutralizing (IL-10) or removing the monocytes abrogated this inhibitory effect. This cereal grain-induced (IL-10) response was polymyxin B sensitive, heat resistant, and inhibited by blocking the Toll-like receptor 4.(Kiyoshi Yamazaki *et al*, 2008).

Serum levels of (IL-10) in Albino rats fed on wheat germ with different treatments and basal diet are presented in table (3). The data revealed that there was significant increase in serum levels of IL-10 in group II (basal diet with 5% of raw wheat germ),III basal diet plus 10% of raw wheat germ), IV ((basal diet with 5% of microwave wheat germ), V (basal diet with 10% of microwave wheat germ) in comparison to group I basal diet (control). . Although level of (IL-10) in groups VI (basal diet with 5% of gamma irradiated wheat germ), VII (basal diet with 10% of gamma irradiated wheat germ), VIII (basal diet with 5% of fermented wheat germ), IX(basal diet with 10% of fermented wheat germ) showed lower levels in comparison to group I but the difference did not reach the level of significance.

This might be due to higher intake of non-nutritive compounds such as lectins. Lectins are typically globular proteins that are resistant to digestion in the gastrointestinal tract. They affect the integrity of the intestinal epithelium and the absorption of dietary antigens, and induce the release of allergic mediators from mast cells in vitro. High dietary intake of lectins such as WGA

may affect the allergic response towards oral antigens in the gut-associated lymphoid tissue. (Watzl, et al.,2001).

Results revealed that the mean serum levels of (IL-10) in groups VI (P <0.05), VII (P<0.01), VIII (P < 0.001), IX (P < 0.001) was significant decrease in comparison to group II. However, a significant increase in level of(IL-10) was observed in group V in comparison to group II (P <0.001).

Results revealed that the mean serum levels of (IL-10) in groups VI (P <0.05), VII (P< 0.001), VIII (P <0.001), IX (P < 0.001) recorded significant decrease in comparison to group III. However, a significant increase in level of IL-10 was observed in group V in comparison to group III (P <0.05).

Plant lectins, such as wheat germ agglutinin constitute common components of the human diet and target the immune system on a daily basis. The wheat germ-deprived diet induced a state of functional unresponsiveness in lymphocytes from primary and secondary lymphoid organs, as evaluated by in vitro stimulation with T cell mitogen phytohaemoagglutinin and B cell mitogen lypopolysaccharides. The unresponsive state on the immune cells colud be reversed by injection of antigen emulsified in oil with inactivated mycobacteria . Dietary signals can thus interact with the immune system possibly influencing its shaping during ontogenesis.(Chignola *et al.*,2002) .Plant lectins are able to modulate important immune mechanisms, including inflammatory reactions and effector functions (Muraille *et al.*,1999).Different diet compositions, which might involve different lectins or different lectin concentrations, had been investigated in terms of their potential influences on human or animal health (Watzl *et al.*, 2001). These and other dietary components might target the immune system on a daily basis and hence should be considered as exogenous immunoregulatory signals. Due to the nutritional requirements of living organisms, dietary signals target the immune system during the whole life of an animal. The immune system might therefore be hypothesized to evolve and shape under the continuous stimulation of dietary signals that might add modulatory effects to those elicited by molecules produced by the immune system itself. If so, the absence of dietary immune-targeting signals might result in a different shaping of the immune system and/or in the alteration of some immune function.

Rats were fed for two generations with a diet containing wheat germ or with the same diet deprived of wheat germ. Wheat germ was chosen as a model foodstuff interacting with the immune system due to its content in wheat germ agglutinin , a lectin known to influence several immune functions in vivo and in vitro(Kilpatrick,1999).as a first general approach, the functional activity of the T- and B-lymphocyte compartments was analyzed in the two groups of rats.

There was significant decrease in level of (IL-10) in groups VI (P <0.001), VII (P<0.001), VIII (P < 0.001), IX (P <0.001) in comparison to group IV. However, a significant increase in level of IL-10 was observed in group V in comparison to group IV (P <0.001).

This might be due to the higher percentage of wheat germ agglutinin given in diets of those groups.

Table (3): Serum levels of interleukin-10 (IL-10) in Albino rats fed on wheat germ with different treatments and basal diet (pg/mL).

Parameters	IL – 10 (pg/mL)	Range
	Mean ± S.D.	
Group I (Basal diet) control	21.00 ± 5.42	15.00 – 29.00
Group II (Basal diet with 5% of RWG)	32.14 ± 5.58 a***	25.00 – 40.00
Group III (Basal diet with 10% RWG)	37.17 ± 7.45 a*** b ^o _o	26.00 – 45.00
Group IV (Basal diet with 5% of MWG)	38.29 ± 8.18 a*** b ^o c ^o	26.00 – 47.00
Group V (Basal diet with 10% of MWG)	44.00 ± 7.85 a*** b*** c* d***	32.00 – 55.00
Group VI (Basal diet with 5% of GWG)	24.00 ± 3.99 a b* c*** d*** e***	20.00 – 30.00
Group VII (Basal diet with 10% of GWG)	23.29 ± 2.87 a ^o b** c*** d*** e*** f ^o	20.00 – 28.00
Group VIII (Basal diet with 5% of FWG)	20.71 ± 5.88 a ^o b*** c*** d*** e*** f ^o g ^o	15.00 – 30.00
Group IX (Basal diet with 10% of FWG)	18.86 ± 4.06 a ^o b*** c*** d*** e*** f ^o g ^o h ^o	14.00 – 25.00

RWG = Raw wheat germ. MWG = Microwave treated wheat germ.
 GWG =Gamma irradiated wheat germ. FWG = fermented wheat germ.

* = P < 0.05 ** = P < 0.01 *** = P < 0.001 ^o : non-significant.

a : Group II, III, IV, V, VI, VII, VIII, IX versus group (control).

b : Group III, IV, V, VI, VII, VIII, IX versus group II.

c : Group IV, V, VI, VII, VIII, IX versus group III.

d : Group V, VI, VII, VIII, IX versus group IV.

e : Group VI, VII, VIII, IX versus group V.

f : Group VII, VIII, IX versus group VI.

g : Group VIII, IX versus group VII

h : Group IX versus group VIII.

There was significant decrease in level of(IL-10) in groups VI (P<0.001),VII(P<0.001), VIII(P< 0.001), IX (P < 0.001) in comparison to group V. The levels of IL-10 in groups VII, VIII, and IX were lower than those of group VI but the difference did not reach the level of significance. The levels of (IL-10) in groups VIII, and IX were lower than those of group VII but the difference did not reach the level of significance.

Fermented wheat germ is a concentrated extract of wheat germ fermented by baker's yeast, used primarily for its ability to stimulate and modulate immune system function. The extract is standardized (formulated to always contain a specific amount of substituted benzo-quinones in a given amount of extract). Results of early research using FWGE in autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) suggest FWGE may be of use in these diseases for its ability to reduce inflammatory cytokines and rebalance the activity of Th1 and Th2 lymphocytes.(Hidvégi .2001).

The potential of oral treatment with AVEEMAR (AVEEMAR), a new benzoquinone-containing fermentation product of wheat germ, on features of experimental systemic lupus erythematosus (SLE) in naive mice, induced by idiopathic manipulation, was studied. We assessed the effect of AVEEMAR on the profile of autoantibody production and the response of Th1/Th2 related cytokines as well as the clinical picture of experimental SLE in the SLE-induced mice. When the product was given in the pre-immunization period, down-regulation of autoantibody production (anti-dsDNA, mouse 16/6 Id, and anti-histones) following treatment with AVEEMAR was noted (eg anti-dsDNA

decreased from 0.898±0.097 OD at 405 nm to 0.519±0.103 OD following treatment). This effect was sustained for at least 4 weeks after discontinuation of the therapy. Serological manifestations associated with a delay in Th2 response (IL-4 and IL-10) were recorded (eg IL-4 decreased from 91.7±8.11 to 59.55±7.78 ng/ml in splenocyte condition media). (Hidvégi .2001).

The levels of IL-10 in groups IX were lower than those of group VIII but the difference did not reach the level of significance. This might be due to the higher percentage of fermented wheat germ given in diets of those groups.

Conclusion

The results of the present study recommend the use of fermented wheat germ as adjunct therapy to augment immune system particularly in immune compromised subject.

REFERENCES

- Abdel Ghafar, N. M. (2004) : Effect of Zinc and vitamin E on immune system of rats. Ph. D Thesis, Faculty of Home Economic, Helwan University.
- Abu-Tarboush, H.M. (1998) : Irradiation inactivation of some antinutritional factors in plant seeds. *J. Agric. Food Chem.*, 46, 2698.
- Abu-Tarboush, H.M. and Ahmed, S.B. (1996): Studies on Karkade (Hibiscus sabdariffa): protease inhibitors, phytate, in vitro protein digestibility and gossypol content. *Food Chem.*, 56, 15.
- Anderson R. (1984) :The immunostimulatory, anti-inflammatory and anti-allergic properties of ascorbate. *Adv Nutr Res.*, 6:19–45 [review].
- Bajaj, K.L. and Kaur, G. (1981): Spectrophotometric determination of L-ascorbic acid in vegetables and fruits, *Analyst* 106, 117-120.
- Bendich, A. (1990): Antioxidant vitamins and their functions in immune responses. *Adv Exp Med Biol*, 262:35-55.
- Boros, L. G; Nichelatti, M and Shoenfeld Y. (2005): Fermented wheat germ extract (Aveamar) in the treatment of cancer and autoimmune diseases, *Ann. N. Y. Acad Sci. Jum.* 1051: 529-42.
- Bowers, J. (2002) : Nutrition and immunity, *You Are What You Eat*. Vol. 11, No. 2.
- Bowers, T. (1997) Nutrition and immunity, Part 2 :the role of selected micronutrients and clinical significance. *Veterinary Clinical Nutrition*, 4:3, 96-101.
- Chew, B.P. (1993) :Role of carotenoids in the immune response. *J Dairy Sci.*; 76: 2804–11.
- Chignola, R.; Rizzi, C; Vincenzi, S; Cestari, T; Brutti, N; Riviera, A. P.; Sartoris, S.; Peruffo, A. D. Band Andrighetto, G. (2002):Effect of dietary wheat germ deprivation on the immune system in Wistar rats: a pilot study 2 (10): 1495-1501.
- Codex Alimentarius Commission (1990): Federal Register Irradiation in the Production, Processing and Handling of Food, Vol. 55, No. 85.
- Delafuente JC, Prendergast JM, and Modigh A. (1986) : Immunologic modulation by vitamin C in the elderly. *Int J Immunopharmacol*, 8:205–11.

- De Waart, F. G, Portengen L, and Doekes G.(1997):Effect of 3 months vitamin E supplementation on indices of the cellular and humoral immune response in elderly subjects. *Br J Nutr*, 78:761-74.
- Diehl, H.F. (1995): *Safety of Irradiated Food*. 2nd edn. Pp . 283-289. New York :Marcel Dekker Inc.
- Diplock, A. (1995) : *Safety of antioxidant vitamins and B-carotein*, *Am J Clin Nutr.*,62(suppl):1510s -1516s.
- Ekstrand, B.; Gangby, I.; Akesson, G. ; Stollman, U. ; Lingnert, H. and Dahl, S. (1993) : *Lipase activity and development of rancidity in oats and ota products related to heat treatment during processing*. *Journal of Cereal Science* ,17, 247-254.
- Farag, M. Diaa El-Din H. (1998): *The nutritive value for chicks of full-fat soybeans irradiated at up to 60 kGy*. *Animal Feed. Sci. Tech.* 73, 319.
- Gerber WF, Lefkowitz SS, Hung CY. (1975): *Effect of ascorbic acid, sodium salicylate, and caffeine on the serum interferon level in response to viral infection*. *Pharmacology*,13:228.
- Glasziou PP and Mackerras D.E.M. (1993): *Vitamin A supplementation in infectious diseases: a meta-analysis*. *BMJ*,306:366–70.
- Hassan, I.A.G. and El-Tinay, A.H. (1995): *Effect of fermentation on tannin content and in vitro protein and starch digestibilities of two sorghum cultivars*. *Food Chern*, 53, 149.
- Hidvégi. M ,(2001): *AVEMAR (a new benzoquinone-containing natural product) administration interferes with the Th2 response in experimental SLE and promotes amelioration of the disease*, Vol. 10, No. 9,622-627.
- Hidvégi, M.; Rásó, E.; Farkas, R. T. ; Lapis, K., and Szende, B. (1999): *Effect of MSC on the immune response of mice*. *Immunopharmacology*, 41, 183-186.
- Hughes, D.A; Wright, A.J.; Finglas, P.M. ;Peerless, A.C.; Bailey A.L.; Astley ,S.B. and Pinder, A.C. (1997): *The effect of beta-carotene supplementation on the immune function of blood monocytes from healthy male nonsmokers*. *J Lab Clin Med* ,129: 309–17.
- Jayaraj, A. P. ; Tovey, F. I. ;Clork, C. g. and Hobsley, M. (2001) : *Gastroent. Hepatol.*, 16 (5), 501.
- Kermasha, S.; Bisakowski, B.; Ramaswamy, H. and Van De Voort, F. (1993): *Comparison of microwave, conventional and combination heat treatments on wheat germ lipase activity*. *International Journal of Food Science and Technology*, 28, 617-623.
- Kilpatrick, D.C. (1999):*Immunological aspects of the potential role of dietary carbohydrates and lectins in human health*. *Eur J Nutr* , 38:107-17.
- Kiyoshi Yamazaki, M.D; Joseph, A. ;Murray ,M. D. and Hirohito Kita M.D.(2008); *Innate immunomodulatory effects of cereal grains through induction of IL-10* .*Journal of Allergy and Clinical Immunology*,121 (1) : 172-178.
- Liu, R. H.,(2007) ;*Whole grain phytochemical and health* .*J. Cereal Sci*,46; 207-219.
- Meydani, S. (1996) : *Effect of (n-3) polyunsaturated fatty acids on cytokine production and their biologic function*, *Nutrition*, 12:S8-S14.

- Meydani, S. N; Barklund, M. P ;Liu, S.; Meydani, M. ;Miller, R.A.; Cannon, J.G. ;Morrow, F.D.; Rocklin, R . and Blumberg, J.B.(1990) : Vitamin E supplementation enhances cell-mediated immunity in healthy elderly subjects. *Am J Clin Nutr* ,52:557-63.
- Mohamed, M. M. H.(2005): Effect of some Egyptian vegetables on the cholesterol in blood serum of white rats. M.Sc.Thesis, Faculty of Specific Education, Ain Shams University.
- Moriguchi, S. and Muraga, M.(2000): vitamin E and immunity ,*Vitamin Horm*, 59 :305-36.
- Muraille, E; Pajak, B.;Urbain, J. and Leo, O. (1999) :Carbohydrate-bearing cell surface receptors involved in innate immunity:interleukin-12 induction by mitogenic and nonmitogenic lectins. *Cell Immunol*,191:1-9.
- Principal Central Lab. of Cairo University, (2008): *Vitamins Assay*, Faculty of Agriculture, Cairo University .
- Ross G.D.; Vetvicka V., Van J., Xia Y. and Vetvicková J. (1999) : Therapeutic intervention with complement and beta-glucan in cancer. *Immunopharmacology*. May;42(1-3):61-74.
- Santos MS, Leka LS, Ribaya-Mercado JD, Russell RM, Meydani M, Hennekens CH, Gaziano JM, Meydani SN. (1997): Short- and long-term beta-carotene supplementation do not influence Tcell-mediated immunity in healthy elderly persons. *Am J Clin Nutr*, 66:917-24.
- Santos, M.S.; Meydani, S. N. ; Leka, L. ; Wu, D. ; Fotouhi, N .; Meydani, M Hennekens C.H., and Gaziano J.M. (1996) : Natural killer cell activity in elderly men is enhanced by beta-carotene supplementation. *Am J Clin Nutr* .64:772-7.
- Scrimshaw, N. (1997) : Synergism or nutrition, infection and immunity: an overview", *Am J Clin Nutr.*, 8(4): 177-83.
- Semba RD. (1994): Vitamin A, immunity, and infection. *Clin Infect Dis*, 19:489-99 [review].
- Sjovall, O.; Virtalaine, T.; Lapvetelainen, A.M. and Kallio, H. (2000): Development of rancidity in wheat germ analyzed by headspace gas chromatography and sensory analysis. *Journal of Agricultural and Food Chemistry*, 48(8): 3522-3527.
- Sukkar, S. G. and E. Rossi (2004) :Oxidative stress and nutritional prevention in autoimmune rheumatic diseases. *Autoimmunity Rev.*, 3:199-206.
- Szent-Györgyi, A., (1982): Biological oxidation and cancer. *Int. J. Quant. Chem. Quant. Biol. Symp.* 9, 27.
- USDA (2006): Oil, wheat germ, National Nutrition Database for Standard Reference, 1.
- W.H.O. (World Health Organization) (1994): Safety and nutritional adequacy of Irradiated Food. WHO, Geneva.
- Wakshull, E. ;Brunke Reese, D. ; Linderemuth, J. ; Fisetite, L .;Nathans, R. S. ; Crowley, J J ; Tufts, J .C. ; Zimmerman, J. ;Mackin, W. and Adams, D. S. (1999) :PGG-glucan, a soluble beta-(1,3)-glucan, enhances the oxidative burst response, microbicidal activity, and activates an NF-kappa B-like factor in human PMN: evidence for a glycosphingolipid beta-(1,3)-glucan receptor.*Immunopharmacology*, Feb; 41(2): 89-107

- Wardlaw, G. (1999) : Perspectives in nutrition.4th ed., Mc Graw-Hill. New York. P. 397, 400-401.
- Watzl, B.; Neudecker, C; Hansch, G.M.;Rechemmer, G.and Pool-Zobel, B. L.(2001): Dietary wheat germ agglutinin modulates ovalbumin-induced immune responses in Brown Norway rat. Br J Nutr., 85 ;483-90.
- Wood ,R. (1999) : The new whole food encyclopedia. New York: Penguin Books.
- Yousif, E. I. (2004): Functional properties and some utilization of wheat germ protein products. Annals of Agricultural Science Cairo, 49 (1) : 139-162.

التأثير المناعي لجنين القمح الخام والمعامل بأشعة جاما والميكروويف و المتخمرفى فئران التجارب.

محمد كمال السيد يوسف* ، فاروق محمد التلاوي** ، نجلاء طه المليجي*** و مشيرة محمد حسن محمد****

* قسم علوم وتكنولوجيا الأغذية - كلية الزراعة - جامعة أسيوط وعضو أكاديمية العلوم الأمريكية.

** قسم الاقتصاد المنزلى - كلية التربية النوعية - جامعة عين شمس.

*** قسم الكيمياء الحيوية الطبية - كلية الطب - جامعة أسيوط

****قسم الاقتصاد المنزلى - كلية التربية النوعية - جامعة أسيوط.

اجريت دراسة بيولوجية على تأثير تناول جنين القمح الخام ، جنين القمح المعامل بأشعة جاما ، جنين القمح المعامل بأشعة الميكروويف ، جنين القمح المتخمر على مستوى {Interferon gamma (IFN- γ) and Interleukin 10 (IL-10)} فى سيرم دم فئران التجارب .

وقد أوضحت النتائج ان تناول جنين القمح المعامل وغير المعامل قد سجل ارتفاعا معنويا فى مستوى (IFN- γ) فى سيرم دم فئران التجارب حيث سجل جنين القمح المتخمر أعلى ارتفاع فى (FN- γ)، ثم جنين القمح المعامل بأشعة جاما ،بلى ذلك جنين القمح المعامل بأشعة الميكروويف وأخيرا جنين القمح الخام مقارنة بالمجموعة الضابطة.كما أوضحت النتائج وجود تغيرات فى مستوى(IL-10) فى سيرم دم فئران التجارب مقارنة بالمجموعة الضابطة وقد سجل جنين القمح المتخمر اقل انخفاض فى مستوى (IL-10) فى سيرم دم فئران التجارب مقارنة بالمجموعة الضابطة. ومن هنا يمكن القول ان جنين القمح غير المعامل و المعامل بالمعاملات السابقة الذكر و خاصة جنين القمح المتخمر يعتبر عاملا منشطا للجهاز المناعى حيث أنه أدى إلى زيادة كفاءة الجهاز المناعى فى فئران التجارب .هذا إلى أن البحث تناول المحتوى الفيتامينى لجنين القمح الخام و المعامل بأشعة جاما و المعامل بأشعة الميكروويف و المتخمر موضحا دور كل منهم فى زيادة كفاءة الجهاز المناعى فى فئران التجارب .

الكلمات المفتاحية: جنين القمح الخام ، جنين القمح المعامل بأشعة جاما ، جنين القمح المعامل بأشعة الميكروويف ، جنين القمح المتخمر ، فيتامينات (A , C , E) ، (IL-10) ، (FN- γ).

قام بتحكيم البحث

أ. د/ محمد طه شلبي

أ. د/ خديجة عبد الحميد حسن

كلية الزراعة - جامعة المنصورة

كلية العلوم - جامعة أسيوط