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Effect of Wheat Germ Extracts on Some Biological Aspects: *In vivo* and *In vitro* Study

A Thesis

Submitted to the Council of Science College / Al- Nahrain University as a partial fulfillment of the requirements for the Degree of Master of Science in Biotechnology

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DEDICATION

To my wonderful family

A special feeling of gratitude to my loving parents , Tawfeq and Salamha Younis whose words of encouragement and push for tenacity ring in my ears, my sister Sadeem and my brother Omar have never left my side and are very special.

I also dedicate this thesis to my uncle Saham Al Selo for his support and to all family members who encourage me and stand beside me

With always love …….

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Summary

This project is aimed to study the inhibition effect of wheat germ extract on some microorganisms, cancer cell line, and atopic dermatitis (eczema).

The quantitative and qualitative detections of methanolic extract and methanol chloroform method extract by using gas chromatography–mass spectrometry (GC-MS) technique were achieved to detect the active groups. Results showed the presence each of linoleic acid (with an percentage of 40%), oleic acid and octadecanoice acid, hexadecanoic acid which might play very important role in all bioactivity evaluations in this project.

The antimicrobial effect of wheat germ extracts (methanol and chloroform methanol) were tested on twelve strains of Gram positive and Gram negative bacteria strains in addition of two fungal strains to find the suitable minimum inhibitory concentration (MIC) the extracts were active and had an antimicrobial activity on almost strains tested MIC ranged between 2.5 to 0.165 mg/ml.The cytotoxicity activity effect of crude extract on cancer human Leukemia cell line (K562), breast cancer cell line (MCF-7) and on normal cell line (Fibroblast).

Results was recorded that a significant cytotoxic activity of the extracts in cell lines during the 72 hr. exposure time at concentrations of (900 , 450, 225 and 112.2) µg/ml as compared to normal cell line fibroblast.

A formula of ointment from wheat germ extracts were prepared and tested topically as a cure treatment for mice with atopic dermatitis (eczema). Both extracts formula; 20% and extract alone proved curing

remarks 0.62 and 0.21 IgE, respectively when compared with beta-methasone 0.05% 0.21 IgE ,after 48 hour .

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List of Abbreviations

Abbreviation	Full name
AD	Atopic dermatitis
Amps	Amphipathic alpha-helical antimicrobial
DMSO	Dimethyl sulphaoxide EDTA
DF	*Dermatofagoid farani*
DMEM	Dulbecco/Vogt modified Eagle's minimal essential *medium*
ELISA	Enzyme-linked immunosorbent assay
GLA	Gamma linoleic acid
GC-MS	Gas chromatography method of separation
IgE	Immunoglobulin E
ICAM-1	Intercellular Adhesion Molecule 1
LPS	Lipopolysaccharides
LA	Linoleic acid
MIC	Minimum inhibitory concentration
MBC	Minimum bacterial concentration
MTT Assay Kit	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
PBS	PBS
Phosphate buffered saline	Phosphate buffered saline
PUFA	PUFA

Poly unsaturated fatty acid	Poly unsaturated fatty acid
RAST	Radioallergosorbent test
RPMI	Roswell Park Memorial Institute medium
ROS	*Reactive oxygen species*
rpm	Rotation per minute
TPA	Tissue plasminogen activator
UVA1	Ultraviolet Authoritative one
WHO	World Health Organization
WGE	Wheat germ extract
WHO	World health organization

1 Introduction and literature review

1.1 Introduction

Natural products are the single most productive source of leads for the development of drugs. Over a 100 new products are in clinical development, particularly as anti-cancer agents and anti-infective (Alan, 2008). Natural products are the source of most of the active ingredients of medicines. This is widely accepted to be true when applied to drug discovery in 'olden times' before the advent of high-throughput screening and the post-genomic era: more than 80% of drug substances were natural products or inspired by a natural compound (Sneader, 1996).

The development of bacterial resistance to presently available antibiotics has necessitated the need to search for new antibacterial agents. Gram positive bacteria such as *Staphylococcus aureus* is mainly responsible for post-operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning (Benayache *et al*., 2001). Gram negative bacterium such as *Escherichia coli* is present in the human intestine and causes lower urinary tract infection, coleocystis or septicaemia (Benjilali *et al*., 1986; Benhassaini et al., 2003). Different antibiotics exercise their inhibitory activity on different pathogenic organisms (Chanda and Rakholiya, 2011). Multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. The development of antibiotic resistance is multifactorial, including the specific nature of the relationship of

bacteria to antibiotics, the usage of antibacterial agent, host characteristics and environmental factors. This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents, but the production cost of synthetic drugs is high and they produce adverse effects compared to plant derived drugs (Abiramasundari *et al*., 2011).

Cancer is 1 of the major health problems (Jemal *et al*., 2004). During the past two decades, the paradigm for cancer treatment has evolved from relatively nonspecific cytotoxic agents to selective, mechanism-based therapeutics. Cancer chemotherapies were initially identified through screens for compounds that killed rapidly-dividing cells. These drugs remain the backbone of current treatment, but they are limited by a narrow therapeutic index. Significant toxicities and frequently acquired resistance. Now, an improved understanding of cancer photogenes has given rise to new treatment options, including targeted agent's and cancer immunotherapy (Ahmedin *et al*, 2007).

Atopic dermatitis, also known as atopic eczema or eczema (Williams, 2009) is a type of dermatitis, an inflammatory, relapsing, non-contagious and itchy skin disorder (Bendetto *et al* ,.2009) Atopic dermatitis is a chronic, highly pruritic (itchy) inflammatory skin disease, and is one of the most common skin disorders in children (Krakowski *et al*., 2008). The disorder results in significant morbidity and adversely affect quality of life (McKenna *et al*., 2008),not only are patients affected by the social stigma of a visible skin condition, but the intense itching

characteristic of the disease often leads to significant sleep disturbances. In addition, management of the condition necessitates the frequent application of emollients (agents that soothe, moisturize and soften the skin) and topical medications, as well as physician visits. Evidence suggests that Atopic dermatitis is a cutaneous manifestation of a systemic disorder that also gives rise to other atopic conditions. In fact, Atopic dermatitis is often the initial step in the "atopic march" (the sequential development of allergic disease manifestations during early childhood), which leads to asthma and/or allergic rhinitis in the majority of afflicted patients (Jayasuriya, 1990) There is no known cure for atopic dermatitis, although treatments may reduce the severity and frequency of flares (Berke , 2012) .

The wheat germ contains riboflavin, thiamine, vitamin E and trace minerals such as zinc (Kumar *et al* .,2001), copper, iron and magnesium (Ranum and Wesley, 2003). Wheat is the best nourishing food that can be easily given to patients and even babies.Wheat has anti-bilious, anti hydrotic, and the antipyretic activity of wheat germ , anti-vinous, sedative, and skin and stomachic properties. Wheat germ oil is a highly rich unrefined oil, the richest sources of vitamin E, A and D (Kumar *et al.*, 2011).

Aim of the study

This study was aimed to investigating

1. Chemical analysis of the wheat germ extract using GC-MS method for both chloroform –methanol, and methanol extract and find the most efficient extract.
2. The anti-microbial activity of wheat germ extracts, on some strains of bacteria and yeast.
3. Studying the anti-cancer activity of the wheat germ extract on the human chronic myelogenous leukemia cell-line (k562) and human breast cancer cell-line MCF-7.
4. Studying the effect of the wheat germ extracts on the atopic dermatitis (eczema) and compare it with chemical drugs in the market used as a treatment.

1.2 Literature review

1.2.1 Wheat

Wheat, a cereal plant of the genus *Triticum* of the family Gramineae, is a major food and an important commodity in the world grain market. It is ranked third in total world production of cereal crops behind coarse grain and maize (Gillin, 2006). It was one of the first grains domesticated by humans (Harlan, 1981). Modern wheat could be classified as soft or hard by gluten content. Flour from hard wheat contains a high percentage of gluten and is used to make bread and fine cakes. The hardest-kernelled wheat is durum (*T. durum*) its flour is used in the manufacture of macaroni, spaghetti, and other pasta products. Soft wheat (e.g. *T. aestivum* var. Rosella) has a high starchy kernel but relatively low gluten level; its flour is preferred for piecrust, biscuits and breakfast foods (Dondlinger, 1908).

The health benefits provided by food products have become a critical marketing tool, primarily because of increasing consumer awareness of the role of food in health promotion and disease prevention. Many epidemiological studies have been carried out on consuming whole wheat. These studies have indicated that whole wheat foods could reduce the risk of cancers (Ferguson and Harris1999; Haggans *et al.*,2000), diabetes (Hallfrisch *et al*, 2000; Venn *et al.,* 2004) and coronary heart disease (Anderson *et al.,2000; ;*Truswell,*2002*). Whole wheat is the intact wheat grain that retains the bran and germ as well as the endosperm. In contrast, refined grains retain only the endosperm. Modern milling of wheat can efficiently separate the bran and germ from the starchy endosperm, which is milled to produce a

finely ground white flour. Whole wheat provides a wide range of nutrients and phytochemicals that optimize health and may have protective effects against cancers, diabetes and coronary heart disease. Components in whole wheat associated with improved health status include lignans, tocotrienols and phenolic compounds, as well as anti-nutrients such as phytic acid and tannins (Slavin, 2003). These components were found to be concentrated in the wheat bran (Bartnik and Jakubczyk 1989).Whole wheat grain consists of the pericarp, the seed coat, the endosperm and germ or embryo (Ferguson and Harris, 1999) (Figure 1.1). Botanically, the term "wheat bran" refers to the outer layers of the wheat grain comprising the aleurone, pericarp and seed coat (Slavin, 2001). In industry, wheat bran is the main by-product of wheat grain milling and grinding and has long been used as a component of various animal feeds. In the last 50 years, there has been greater awareness of the biological activities associated with wheat bran in the prevention of diseases and promoting health for humans.

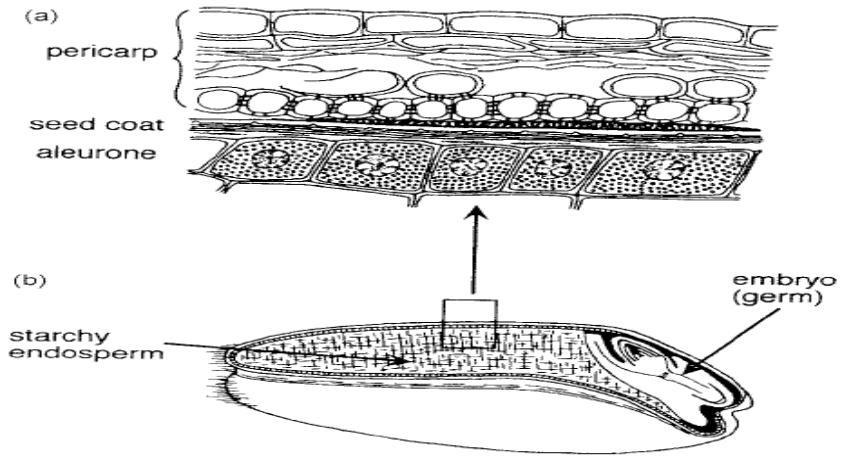


Figure 1.1. Structure of wheat grain and wheat bran.
(a) Longitudinal section of the bran layers of a wheat grain showing the different cell types. (b) Longitudinal section through a wheat grain: the rectangle indicates the location of the section shown in (a)((Ferguson and Harris, 1999).

1.2.2 Wheat health benefits

The phytochemicals present in grains are believed to protect against some types of cancer, cardiovascular disease, diabetes and obesity (Slavin, 2003; Jones *et al*., 2004). Whole grains are even postulated to deliver more phytochemicals and antioxidants than many of the fruits and vegetables (Jones *et al*., 2004). These health benefits are achieved through multifactorial physiological mechanisms including antioxidant activity, mediation of hormones, enhancement of the immune system and facilitation of substance transit through the digestive tract, butyric acid production in the colon, and absorption and/or dilution of substances in the gut (Adom *et al*., 2005).

1.2.3 Composition of a wheat seed

Because wheat has become a staple source of protein and calories for both animals and humans, the chemistry of the wheat plant has been known since the early1900s (Osborne and Harris, 1906).

The wheat kernel or seed is structured to provide the maximum amount of nutrition and Protection to the embryo (germ). In order to pass along Genetic material, germination must occur and the wheat plant must ensure the seed is equipped with sufficient nutrition to support, growth. The food reserves for the embryo are located in the endosperm and Consist primarily of starch (20-30% amylose and 70- 80% amylopectin) and proteins (12%) (Srivastava, 2002), whereas the embryo itself makes Up approximately 3% of the entire grain and consists of concentrated nutrient sources with approximate ratios of 28% protein, 12% lipid, 30% Starch, and 24% fiber (Hernot *et al*., 2008). Since the agricultural revolution, humans have come to rely on the

concentrated food reserves stored in seed endosperms. Wheat, rice, and maize seeds supply approximately 50 % of all food Calories consumed by humans (Biodiversity International, 2008). To enhance the calories available for exploitation, seed sizes have been dramatically increased beyond their wild-type size through selective Breeding techniques (Srivastava, 2002). Because the proteins in seeds are synthesized for use specifically by the developing embryo, the Composition is unique from those proteins found in the vegetative parts of the plant. Seed proteins are contained in a membrane-bound protein storage vacuoles and are classified, based on their solubility, into four groups (Srivastava, 2002):

a. Albumins: soluble in water or dilute buffers at neutral pH
b. Globulins (legumins and vicilins): soluble in salt solutions
c. Prolamins: soluble in alcohols (70-90% solution)
d. Glutelins: soluble in dilute acids or alkalis .

As early as 1906, scientists had characterized the proteins of the wheat Kernel and diagrammed their location. Of significance, gliadin and glutenin, a prolamin and a glutelin, respectively, were identified as the primary proteins present in the endosperm, whereas globulin and albumin proteins appeared to be contained solely within the embryo (Osborne & Harris, 1906). In the Production of white flour, the starches and proteins of the endosperm are separated from the germ and bran and pulverized into a fine powder. Gliadin comprises the majority of protein in the endosperm and due to its dense interchain disulfide bonds, has the unique ability to form gluten upon the addition of water—a coherent, elastic mass that remains after the starch and water soluble proteins have been eliminated (Srivastava, 2002). A staple for the baking industry, the

composition of flours are also important for health reasons. Increasingly, gluten-free products are Requested in an attempt to avoid the gliadin fraction, the protein Responsible for symptoms associated with celiac disease (Drago *et al.,* 2006). On average, the protein composition of typical Flours appears to be 10%:40%:48% albumin/globulin: gliadin:glutenin (DuPont *et al.,* 2005). Additionally, the soil characteristics affect the final nutrient composition of the wheat prior to milling. The availability of nitrogen and sulfur during seed filling for instance, might favor starch or oil deposition over Proteins affecting the final nutrient profile of the wheat Seed (Srivastava, 2002).

1.2.4 Wheat germ

Wheat germ is a by-product of the wheat milling industry, germ constitutes about 2-3% of the wheat grain and can be separated in a fairly pure form from the grain during the milling process (Krings *et al,* 2001*).* Wheat germ contains about 11% oil (Dunford *et al.,* 2003), wheat germ oil is used in products such as foods, biological insect control agents, pharmaceuticals and cosmetic formulations (Kahlonet, 1989). Polyunsaturated fatty acids and bioactive compounds are prone to oxidation and degradation under the conditions used for conventional edible oil extraction and refining methods (Krings *et al.,* 2000) ,the germ contains riboflavin, thiamine, vitamin E and trace minerals Such as zinc, copper, iron and magnesium (Kumar *et al*., 2011).

Wheat is the best nourishing food that can be easily given to patients and even babies (Kumar *et al.,* 2011) , a 2008 study in rats showed that rats given wheat germ had significantly higher protective levels of vitamin E in their blood and liver, conferring greater anti-oxidant protection

(Leenhardt *et al* ., 2008). Other studies showed a decrease in recurrence of melanoma (a Very difficult to treat form of skin cancer) in patients who were using Chemotherapy, and helped raise white counts and lower fevers in kids Undergoing chemotherapy (Garami *et al.,* 2004). A study suggested that the fermented wheat germ was able to reduce oxidative stress in patients with head and neck cancers, and improve these patients' quality of life (Sukkar *et al* ., 2008).

Wheat germ is sodium and cholesterol free, and dense in nutrients. It is rich in vitamin E, magnesium, pantothenic acid, phosphorus, thiamin, niacin and zinc. It is also a source of coenzyme Q10 (ubiquinone) and PABA (para-amino benzoic acid), (Shewry, 2009).

Wheat germ is also high in fiber, and contains approximately 1 gram of fiber per tablespoon. A diet high in fiber can be useful in regulating bowel function (i.e. reducing constipation), and may be recommended for patients at risk for colon disease, heart disease, and diabetes. Wheat germ and wheat bran can be a good source of dietary fiber helping in the prevention and treatment of some digestive disorders (Simmonds, 1989). The antioxidant activity and phytochemical content were studied in milled grain of eleven varieties which included a range of red and white wheat and durum wheat. Whole-wheat bread is good for health. There is no doubt that the adaptability and high yields of wheat have contributed to its success, but these alone are not sufficient to account for its current dominance over much of the temperate world.

1.2.5 Chemical composition of wheat germ

Fats and oils play an important role in the food industry and are essential part of human nutrition. Oils provide fat-soluble vitamins such as vitamins A, D, E, and K, and are also source of essential unsaturated fatty acids, which cannot be synthesized by the human body. In order to meet nutritional requirements, new vegetable oil resources are being

sought as sources of these vitamins and essential fatty acids (Piras *et al.*, 2009).

Wheat germ oil has the highest tocopherol content of all vegetable oils, up to about 2,500 mg/kg, and also the highest content of α-tocopherol, which represents around 60% of the total content (Shuler, 1990). Both of which are of great importance in human metabolism and cannot be synthesized by the organism. They are precursors of a group of hormones called prostaglandins, which play an important role in muscle contractions and in the proper healing of inflammatory processes (Coultate, 1989). Furthermore, linoleic acid helps to eliminate cholesterol and is a precursor of cell membrane phospholipids (Salinas, 1993).

Solvent extraction is a common method of extraction of oils from vegetable matter. The study of the best method of extraction for the wheat germ they found that methanol extraction is reported that the yield of oil is much higher than other extraction methods and the yield of α-tocopherol extracted by the methanol chloroform extraction were much higher than those prepared by chloroform/methanol extraction (Figure 1.2). However, the olution of chloroform/methanol showed a stronger solvent power to extract α -tocopherol form wheat (Piras *et al* , 2009).

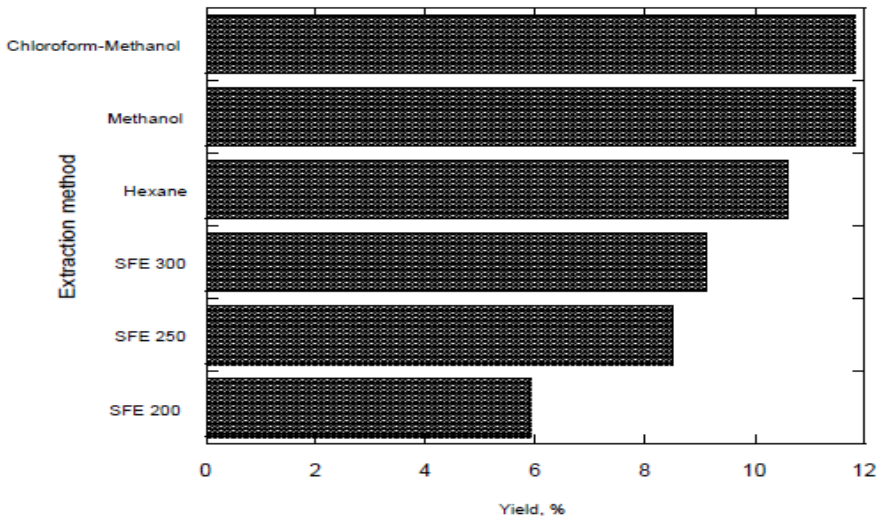


Figure 1.2: Effect of extraction method on the yield of wheat germ oil (Piras *et al*., 2009).

1.2.6.1 α-Tocopherol

1.3.6 Tocopherols

Is a form of vitamin E that is preferentially absorbed and accumulated in humans (Rigotti, 2007), there are three stereo centers in alpha-tocopherol, so this is a chiral molecule (Jensen and Lauridsen, 2007).

Wheat germ oil include a plenty of vitamin E which is a natural, highly tolerable and cost effective molecule. This generic term is used for tocopherol and tocotrienols consisting of two rings with a hydrocarbon chain.Both structures are similar, although the tocotrienol structure has double bonds on the isoprenoid units. Natural vitamin Es are known as α, β, γ, and δ according to the methyl or proton groups that are bound to their benzene rings, and the most common and biologically active form is alpha-tocopherol (Brigelius and Traber, 1999) when produced synthetically, it is composed of eight stereoisomers in which RRR-α-tocopherol is the most biologically active form (Helzlsouer *et al.,* 2000).

Beyond the nonspecific antioxidant effect: Specific effects of vitamin E, which includes gene regulation, have been revealed, and non-antioxidant properties of tocopherols are topics of interest (Azzi and Stocker, 2000). In many *in vivo* and *in vitro* studies, the anti-proliferative effect vitamin E has been shown (Tran *et al.,* 1990; Traber MG *et al.,* 1995). Protein kinase C (PKC) is one of the pathways used by α-tocopherol (Boscoboinik *et al.,* 1991). Sharma with his group reported that tocopherolinhibits not only free radical formation but also tyrosine kinase activity in Tissue Plasminogen Activator (TPA)-induced primary human fibroblasts or HL-60 cells. Results of many published *in vivo* and *in vitro* collaborative studies illuminate the anti-proliferative effect of α-

tocopherol via the PKC pathway in the vascular smooth muscle cell model (Sharma *et al* ., 1994).

1.2.6.2 Linoleic acid

Wheat germ is rich in Linoleic acid, an omega-6 fatty acid (Janet, 2012). The body uses linoleic acid to produce gamma linoleic acid, via conversion exhibits anti-inflammatory, antithrombotic, anti-proliferative, and lipid-lowering potential. It also enhances smooth muscle relaxation and vasodilation. Gama linoleic acid has been promoted as medication for a variety of ailments including breast pain and eczema, in particular by David Horrobin (1939 – 2003), whose marketing of evening primrose oil was described by the British Medical Journal as ethically dubious – the substance was likely to be remembered as "a remedy for which there is no disease", In 2002 the UK's Medicines and Healthcare products (Richmond, 2003).

Regulatory Agency withdrew marketing authorisations for evening primrose oil as an eczema remedy (Williams, 2003). The *British Medical Journal* commented in 2003 that it had taken 20 years to demonstrate that the substance was of no use in atopic dermatitis, and called for more transparency in the research on which drug licensing decisions were taken (Smith, 2003).

Gama linoleic acid is also sometimes promoted as an anti-cancer agent. According to the American Cancer Society there is very little evidence for its effectiveness, and "neither gamma linoleic acid nor other gamma linoleic acid -rich supplements (such as evening primrose oil) have been convincingly shown to be useful in preventing or treating any other health conditions (American Cancer Society, 2013), Alterations in linoleic acid

metabolism have been demonstrated in atopic conditions such as eczema (Melnik and Plewig, 1989).

Conversion of linoleic acid to gamma linoleic acid is inhibited in individuals with atopic dermatitis. During the past two decades several studies have reported mixed results on the use of gamma linoleic acid - containing oils, particularly Erythropoietin, for atopic (Andreassi, 1997) (Henz *et al.*, 1999), these studies reveal subtle improvements, such as decreased inflammation and itching; however, overall numbers failed to reveal significant change.

In a multicenter trial, 179 patients with atopic dermatitis were treated with 4 g erythropoietin daily. After 12 weeks, 62 percent of patients demonstrated a significant clinical response based on a standardized clinical assessment form (Steward *et al.*, 1991). Wright and Burton treated 60 adults and 39 children with atopic eczema with erythropoietin or placebo for 12 weeks,27 adults received either 1.44 g erythropoietin (180 mg gamma linoleic acid) or two times or four times that dose. Children received either 0.72 g erythropoietin (90 mg gamma linoleic acid) or two times that dose. A moderate improvement in clinical signs, including itching, followed supplementation, particularly at the highest doses of erythropoietin most recently a double-blind, randomized, placebo-controlled trial was conducted on 118 formula- fed infants who were at high familial risk of developing atopic dermatitis.(Van Gool *et al* ., 2003). Infants with a maternal history of atopic disease received a borage oil supplement (100 mg gamma linoleic acid) or placebo (sunflower oil) daily for the first six months of life. Outcome was based on incidence and severity of atopic dermatitis as well as total serum immunoglobulin E (IgE). Clinically, severity of atopic dermatitis was decreased favorably in the borage-oil group, although atopy was still

present. Additionally, gamma linoleic acid had no effect on IgE levels during the first year (Bakshi *et al*., 2003)

1.2.6.3 Oleic acid

Epidemiological studies have suggested a positive association between the total fat intake and the risk of cancer, particularly breast, colorectal and prostate cancers (Zock, 2001). Carcinogenesis models have also provided evidence for a lipid specific action beyond their caloric supply, thus suggesting that the type of fat and its unique composition are of greater importance than overall fat intake (Wiseman ,2008), (Kushi and Giovannucci, 2002).

Whereas a high intake of n-6 polyunsaturated fatty acids (PUFA) has tumor-enhancing effects, n-3 PUFA have inhibitory effects. However, there are few experimental studies addressing the role of monounsaturated fatty acids of the n-9 family, such as oleic acid, on cancer, if compared to the investigations about the role of other dietary lipids. Oleic acid has attracted much attention, especially in the last few years, as the "Mediterranean diet", characterized by a high olive oil (rich in oleic acid) consumption, has been traditionally linked to a protective effect against cancer. A wide range of studies have been conducted into breast cancer, where a potential protective effect of olive oil and oleic acid has been described. In addition, epidemiological studies suggest that olive oil may have a protective effect on colorectal cancer development. Some animal studies have also shown that dietary olive oil prevented the development of colon carcinomas in rats, corroborating that olive oil may have chemo preventive properties against colon carcinogenesis (Bartsch *et al* ., 2002).

Finally, a novel approach to chemotherapy has the potential to yield novel dietary-drug combinations that can provide additive or even synergistic protection against the progression of cancer and it is especially relevant when the etiology of disease development has varied mechanistic routes. With regard to this novel approach, oleic acid has been reported to act synergistically with cytotoxic drugs, thus enhancing their antitumor effect, Thus, both epidemiological and animal studies have reported a protective role of oleic acid in several cancers. However, the mechanisms behind the antitumor effect of such a fatty acid are not well understood. One of oleic acid uses as as emollient (Carrasco, 2009).

1.3 Anti-microbial activity of wheat

The first antibacterial peptide isolated from a plant species was a purothionin from wheat flour (*Triticum aestivum*), which has the ability to inhibit the growth of some phytopathogens such as *PseudomonasSolanacearum, Xanthomonas*
campestris and Corynebacterium michiganense (Fernandez *et al.*, 1972). Almost 40 years later, several additional peptides with antibacterial activity have been characterized, represented not only by thionins, now named defensins, but also by other groups of proteins such as cyclotides, glycine-rich proteins, snakins, 2S albumins, and hevein-type proteins (Claudeand Selitrennikoff 2001; Daly *et al.*, 1999). Peptides have been isolated from roots, seeds, flowers, stems, and leaves and have demonstrated activities towards phytopathogens, as well as against bacteria pathogenic to humans (Pelegrini *et al.*, 2005). Over the years, antibacterial peptides have become an interesting tool for the development of new techniques in the control of crop losses and in the production

of novel antibiotics for the treatment of diverse human infections (Belk *et al*., 1999; Pelegrini *et al*., 2008).

However, there is still little information about how these peptides affect the pathogen to cause cell death or growth inhibition. The fact that only a few peptide structures have been studied makes it more difficult to clarify the mechanism of action used to cause damage in bacterial cells (Saether *et al.*, 1995; Daly *et al.*, 1999). Furthermore, it is not clear whether plant antibacterial peptides from different protein families present similar sequences, structures, and modes of action, or whether each group behaves in a different manner.

Accordingly, the study intends to explain some of these features of antibacterial peptides from plant sources. Herein, biochemical and structural properties of several peptides from different protein groups demonstrating antibacterial activity are described. Three-dimensional structures already obtained for plant antibacterial peptides are evaluated, and some possible mechanisms of action including cell membrane disruption, growth inhibition, and death are also proposed. Finally, the similarities among many antibacterial peptides are compared, and their most conserved attributes are evaluated (Hancock and Sahl, 2006).

Until now, there have been few reports about the mechanism of action of plant antibacterial peptides. However, these molecules have some important features responsible for antimicrobial activity, including their amphipathic structures and cationic charge at physiological pH (Epand and Vogel, 1999; Yeamn and Yount, 2003). The main hypothesis for their mechanism of action involves the ability of amphipathic alpha-

helical antimicrobial (AMPs) peptides to cause membrane collapse by interacting with lipid molecules on the bacterial cell surface .According to this hypothesis, the cationic peptides are attracted electrostatically to negatively charged molecules such as anionic phospholipids, lipopolysaccharides (LPS) (Gram-negative) and teichoic acid (Gram-positive), which are located asymmetrically in the membrane architecture.

The positively charged residues can also interact with membrane lipids through specific receptors at the surface of the cell (consequently, peptide binding to the membrane can activate several pathways that will cause cell death (Sitaram and Nagaraj, 1999).

However, one general mechanism of action for antibacterial peptides is observed for most peptides. When they reach a threshold concentration, cationic peptides accumulate on the membrane surface in order to direct inner targets for cell lyses. Intrinsic and extrinsic parameters have been reported to influence the threshold peptide concentration. Intrinsic factors include the ability of the peptides to self-assemble and oligomerize, while extrinsic determinants include phospholipid membrane composition, membrane fluidity and head group size; these factors all influence membrane potential, which is critical for determining the threshold peptide concentration (Shai ,2002).

The following three processes of pore formation have been reported for plant antibacterial peptides: the barrel-stave mechanism, the toroid pore or wormhole mechanism and the carpet mechanism (Padovan *et al.*, 2010). The barrel-stave

mechanism consists of peptide aggregates forming a barrel-ring around an aqueous pore. Peptides interact with the membrane, forcing one thin and hydrophobic portion to bind the phospholipid acyl-chains. After reaching threshold concentration, peptides from the barrel-ring open a pore in the membrane. Their hydrophilic portions comprise the core of the barrel, while the hydrophobic portion interacts with bacterial membrane phospholipids (Shai, 2002).

1.4 Wheat germ anti-cancer activity

Fermented wheat germ is the fermented wheat germ extract is produced from wheat germs of the genus *Triticum vulgaris*, the active ingredient in fermented wheat germ extract is not yet known, It has been claimed that orally used fermented wheat germ extract offers beneficial effects for cancer patients during chemoand / or radiotherapy. The evidence from clinical trials to support claims of efficacy is weak. The oral intake of fermented wheat germ seems to cause no harm. Fermented wheat germ is often falsely advertised as a cure for cancer, as some believe, studies have shown that is has cured cancer in some cases (Boros *et al.,* 2001).

Fermented wheat germ extract (wheat germ extract) was developed in the 1990s by Hungarian chemist Mate Hidvegi. It should not be confused with wheat germ oil. WGE is used as a dietary supplement by cancer patients in Hungary to improve quality of life.

Results from in vitro studies show that WGE has anticancer (Saiko *et al.,* 2007), anti-metastatic (Hidvegi *et al.,* 1998), and

immune modulatory effects (Jakab, *et al;* 2003; Taube *et al.*, 2008) . Although it appears to increase estrogen receptor (ER) activity, wheat germ extract enhanced efficacy of tamoxifen, an ER antagonist, in ER+ breast cancer cells (Marcsek *et al;* 2004) as well as cisplatin in ovarian cancer cell lines (Judson *et al;* 2012). Animal models suggest wheat germ extract can reduce cardiovascular symptoms due to chronic hypertension, diabetes, and obesity (Iyer *et al;* 2011), mitigate symptoms associated with lupus (EhrenfeldM *et al;* 2001), and that its antitumor effect is comparable to other endocrine treatments (Tejeda *et al;* 2007).

Data from pilot studies indicate a beneficial role for wheat germ extract in patients with colorectal cancer (Jakab *et al.*, 2003) and in reducing treatment-associated febrile neutropenia in pediatric cancer patients (Garami *et al.*, 2004),It also prolonged survival of patients with melanoma when used with chemotherapy . However, these effects must be confirmed by large-scale, well-designed clinical trials (Telekes *et al.*, 2005; Demidov *et al.*, 2008).

Because it potentiates estrogen receptor activity, patients with hormone-sensitive cancers should use wheat germ extract with caution (Garami *et al.*, 2004).

1.4.1 Mechanism of Action

Benzoquinone compounds are thought to be the active components of wheat germ extract (Judson *et al;* 2012). In vitro, wheat germ extract attenuates cell cycle progression from G2-M to G0-G1 phase, reduces ribonucleotide reductase activity (Saiko *et al;* 2007), and stimulates

immune function via increased natural killer (NK) cell activity and intercellular adhesion molecule 1 (ICAM-1) expression (Fajka-Boja *et al.*, 2002). It also increases tumor necrosis factor (TNF) and cytokine production by activating metabolic pathways involved in tumor cell death (Telekes *et al;* 2005). Wheat germ extract demonstrated cytotoxic effects on human lymphoma cells by inducing apoptosis (Saiko *et al;*, 2003) and against ovarian cancer cells via poly (ADP-ribose) polymerase (PARP)-1 and PARP-12 expression (udson *et al;* 2012). It was also shown to regulate tumor cell proliferation by inhibiting glycolysis and pentose cycle enzymes, and induce apoptosis through caspase-3-mediated PARP cleavage (Judson *et al .,* 2012).

Their study was done to determine if wheat germ extract supplementation was beneficial for patients with colorectal cancer. A total of 66 patients were given wheat germ extract 9g once daily in addition to anticancer treatments, and 104 patients received only anticancer treatments. Data analysis revealed that patients who took wheat germ extract less few disease progression-related events compared with the control group. There was also an improvement in OS of patients on wheat germ extract, but more trials are needed with equal numbers of patients in the experimental and control groups (Jakab *et al .,2003*).

1.5 Leukemia

A group of cancers that usually begins in the bone marrow and results in abnormal blood cells in high numbers (NCI, 2004). It is characterized by an increase in immature white blood cells called blasts. It including: acute lymphoblastic leukemia, acute myeloid leukemia, chronic lymphocytic leukemia and chronic myeloid

leukemia among others (Colvin and Elfenbein , 2014) in turn, it is part of a broader group cancers which affect the blood, bone marrow, and lymphoid system, known as hematological malignancies, While the exact cause of leukemia is typically unknown, a combination of environmental and genetic factors are believed to play a role some risk factors include: Down syndrome (NCI, 2013).

Leukemia is a treatable disease. This may involve some combination of chemotherapy, medical radiation therapy, hormone treatments, or bone marrow transplant. The rate of cure depends on the type of leukemia as well as the age of the person. Children are more likely to be permanently cured than adults. Even when a complete cure is unlikely, most people with a chronic leukemia and many people with an acute leukemia can be successfully treated for years. Sometimes, leukemia is the effect of another cancer, known as blastic leukemia, which usually involves the same treatment, although it is usually unsuccessful. Outcomes have improved in the developed world due to treatment , in 2012 leukemia developed in 352,000 people and cause 265,000 deaths (WHO, 2014).

About 90% of all leukemias are diagnosed in adults (Hutter, 2010) it occurs more commonly in the developed world (WHO, 2014) .

1.6 Atopic dermatitis

Also known as atopic eczema or eczema is a type of dermatitis, an inflammatory, relapsing, non-contagious and itchy skin disorder atopic dermatitis is generally considered idiopathic, that is, without a known cause (kim, 2012) Although a genetic component is suspected, as is supported by the finding that many persons with atopic dermatitis have a

family history of atopy, which are immediate-ons*et al;*lergic reactions such as asthma, food allergies, atopic dermatts or hay fever(Varothai *et al;* 2013) In 2006 it was discovered that mutations in the gene for the production of filaggrin strongly increased the risk for developing atopic dermatitis. Most importantly two mutations were found that affect approximately 5% of people in Western Europe that may disrupt the production of filaggrin. Filaggrin is a protein that plays an important role in the retention of water in the stratum corneum. People who have these mutations often have dermatitis (Yarbrough *et al.,* 2013), Likewise children with poor hygiene are at a lower risk for developing atopic dermatitis , as are children who drink unpasteurized milk Exposure to dust mites are believed to contribute to one's risk of developing atopic dermatitis (fuino and incrovia, 2012), A diet high in fruits seems to have a protective effect against atopic dermatitis , whereas the opposite seems to be true for fast foods(Carsten *et al.*, 2014).

There is no known cure for atopic dermatitis, although treatments may reduce the severity and frequency of flares (Berke *et al.*,2012) applying moisturisers may prevent the skin from drying out and decrease the need for other medications (Varothai *et al.*, 2013) Affected persons often report that improvement of skin hydration parallels with improvement in atopic dermatitis symptoms (Berke *et al.*, 2012).

Additionally topical corticosteroids, especially hydrocortisone have proven themselves effective in managing atopic dermatitis (Berke *et al;* 2012 ; Kim, 2014) If topical corticosteroids and moisturisers fail, short-term treatment with topical calcineurin inhibitors like tacrolimus or pimecrolimus may be tried, although they are usually avoided as they can cause skin cancer or lymphoma Alternatively systemic immunosuppressants may be tried

such as ciclosporin, methotrexate, interferon gamma-1b, mycophenolate mofetil and azathioprine. (Berke *et al;* 2013; Yarbrough *et al;* 2013) Antidepressants and naltrexone may be used to control pruritus (itchiness) (Kim, 2012). A more novel form of treatment involves exposure to broad or narrow-band ultraviolet light. UV radiation exposure has been found to have a localized immune modulatory effect on affected tissues and may be used to decrease the severity and frequency of flares (Tintle *et al;* 2011; Beattie *et al;* 2005) In particular, Meduri *et al;*. have suggested that the usage of UVA1 is more effective in treating acute flares, whereas narrow-band UVB is more effective in long-term management scenarios (Meduri *et al*., 2007) However, UV radiation has also been implicated in various types of skin cancer, and thus UV treatment is not without risk (Jans *et al;* 2006).

Table (1.1): The compounds in wheat germ and there bioactivity.

No	Name of the compounend	Name	Bioactivity
1	Hexadecanoic acid, ethyl ester	palmitic acid ester	Antioxidant,Hypocholesterolemic Nematicide, Pesticide,Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor
2	9,12-Octadecadienoic acid (Z,Z)-	Linoleic acid	Anti-inflammatory Hypocholesterolemic Cancer preventive Hepatoprotective Nematicide Insectifuge, Antihistaminic Antieczemic Antiacne, 5-Alpha reductase inhibitor Antiandrogenic Antiarthritic Anticoronary Insectifuge
3	Vitamin E	Vitamin E compounds	Antiageing,Analgesic,Antidiabatic, Antiinflammatory, Antioxidant, Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Hypocholesterolemic,Antiulcerogenic, Vasodilator, Antispasmodic, Antibronchitic, Anticoronary
*	**Source: Dr.Duke's: Phytochemical and Ethnobotanical Databases**		

2.1 Materials and methods

2.1.1Apparatus and equipment

The following apparatus and equipment were employed in the present study were listed below:

Table (2.1): Equipment's and their origin.

Apparatus	**Company / Origin**
Autoclave	HIRAYAMAHV/ Japan
Automatic ELISA test workstation	Crocodile/USA
Animal isolator unit	Transit / England
Auto pipette	LABFREEZ/China
Centrifuge (Portable)	Eppendorf / Germany
Corning 24- well Multiwell Plates	Sigma
Corning 96-Well Plates Multiwell plates	Sigma
Digital camera	HTC 18 mega /Taiwan
Filter-cap culture flasks	NunClon/ Denmark
GC-MS	WatersMicromass /USA
Incubator	IKS / England
Laminar air flow cabinet	Lambart/ England
Micropipette	Eppendorf
PH meter	Veolia /France
Rotary evaporator	EV LAB TECH / USA
Spectrophotometer	ATI UNICAM /USA
Water Bath shaker	KSR/ South Korea
Whattman filter paper	Sigma

2.1.2: Chemicals and media used

Table (2.2): Chemicals and kits used in this study and their suppliers.

Chemical	**Company / Origin**
Chloroform	Sigma Aldrich /USA
Dimethyl sulfoxide (*DMSO*) Normal grade	Sigma Aldrich , USA
Dimethyl sulfoxide (*DMSO*) Tissue culture grade	Sigma Aldrich USA
Dermatophagoides (house dust mite)	Greer Laboratories/USA
Ethanol	Sigma Aldrich /USA
Either	Sigma Aldrich /USA
FBS	HyClone, USA
Hite Petrolatum 95 %	Fougera / USA
HEPES	Lonza, USA
Methanol	Sigma Aldrich /USA
MTT assay Kit	Promega, USA
Propylene Glycol (SP Gr = 1.035)	Sigma Aldrich /USA
Stearyl Alcohol %	Sigma Aldrich / USA
Sodium Lauryl Sulfate	Sigma Aldrich /USA
Typsin	Sigma Aldrich / USA
White Wax 5%	Osmo / USA

Table (2.3): Culture Media

Medium	**Company / Origin**
Brain heart infusion agar	Bio lab , Hungary
Brain heart infusion broth	Bio lab , Hungary
Dulbecco's Modified Eagle Medium (DMEM)	Gibco® / USA
Mular Hinton Agar	Bio lab / Hungary
Nutrient Agar	Bio lab / Hungary
Roswell Park Memorial Institute medium	Gibco® / USA

Table (2.4): Cell line cultures

Cell line	**Company / Origin**
Fibroblast cells	American Tissue Culture Collection /USA
Leukemia k562 cells	American Tissue Culture Collection /USA
MCF-7 cells	American Tissue Culture Collection /USA

2.1.3 Wheat germ sample collection

Wheat germ was obtained from (Al Doraha Mill Company, Iraq), and was stored at room temperature (20 – 25 ˚C) until the time of extraction.

2.1.4 Bacteria and Yeasts

Bacterial and yeast strains are listed in table (2.5) below , all strains were obtained from biotechnology and genetic engineering department Philadelphia university in Jordan, and were maintained on Brain Heart Infusion (BHI) agar medium at 4 ºC.

2.1.4 Culture medium

Types of media were required for carrying out this study, Brain Heart Infusion broth, nutrient agar, and Mueller-Hinton agar media, all media, were sterilized by autoclaving at 121 ºC (15 Ib/in^2) for 2 hours, volumetric flasks were used and agar media were left to solidification and broth media were kept at the cold room at 3 ºC till the time of use. This experiment was carried out at Biotechnology dep.at Philadelphia University, Jordan.

Table (2.5): Bacteria and yeast strains (American type culture collection) /ATTC, USA.

Se.	Type of microorganism	Name of Organism	ATCC number (American tissue culture collection)
1	Bacteria	*Staphylococcus aureus*	ATCC 1927
2		*Escherichia coli*	ATCC 2593
3		*Salmonella typhimurium*	ATCC 2054
4		*Bacillus cereus*	ATCC 1014
5		*Klebsiella pneumonia*	ATCC 1705
6		*Staphylococcus epidermidis*	ATCC 12228
7		*pseudomonas aeruginosa*	ATCC 9027
8		*Enterococcus faecalis*	ATCC 29212
9		*serratia marcescens*	ATCC 13880
10		*micrococcus luteus*	ATCC 9341
11	Yeast	*candida albicans*	ATCC 90028
12	Yeast	*candida tropicalis*	ATCC 18754

2.2 Methods

2.2.1 Preparation of plant extracts

Rotary evaporator was used for extraction. Wheat germ samples (63) g were putt for overnight in a shaking water path at 40 ˚C, filtered through Whatman papers 90mm diameter from (sigma Aldrich), then were placed into the rotary evaporator for 3 hours at 40 C until the methanol and methanol chloroform was totally evaporated from the sample, and the extracts were gently putt into flacks and were stored in a dark at room temperature (Alessandra *et al*.,2009).

a. Methanol extract

A portion of 400 ml of methanol (95 %)HPLC grade solution were added to 63 g of air- dried wheat germ, at room temperature in order to avoid fatty acids oxidation and physically removed particulates.

b. Chloroform methanol extract

The procedure of 2.2.1.a was used expect methanol: chloroform (50:50) used in the extraction instead of the methanol alone.

2.2.1.1 Preparation of stock aqueous wheat germ extract

Stock aqueous extract of wheat germ was prepared by mixing 0.1g of wheat germ with 10 ml of (17.5 ml sterile distilled water, and 2.5 ml of DiMethyl Sulphoxide DMSO) to obtain 10 mg/ml of stock solution.
Different concentration were prepared from the stock solution and filtered by 0.22μm membrane filters.

2.2.1.2 GC-MS analysis experiment

Samples of wheat germ sent to Millis Scientific, Inc., (Maryland, United States) for GC-MS analysis. A quantity of 0.4 g of wheat germ was mixed with 4 ml of methanol-chloroform (1:1), incubated for 24hr. at 30C, supernatant was decanted, centrifuged, evaporated to about ½ of initial volume in vacuum and used for analysis.Waters/Micromass Quatro GC mass spectrometer interfaced to a Thermo Electron TraceGC gas chromatograph was utilized for the analysis the mass spectrometer was set to scan ions in the 45-350 Da range over 0.5 sec. Ion source temperature was 180°C and that of transfer line was 320°C.
The GC was equipped with a DB-1 30m length, 0.25 mm ID column. Helium was used a carrier gas with the flow rate of 1 ml/min. The

injector temperature was 260°C and the split ratio was 10, 1 ul was injected. The GC oven temperature profile was as follows: Initial temperature was 100°C, held for 2 min, ramp 10 C/min to reach 320°C, held for 5 min at this final T.NIST 05 mass spectral library was used to identify eluting compounds by matching their mass spectra with those referenced in the database. The database catalogs more than 163.000 electron impact (EI) mass spectra.

2.2.2 Anti-Microbial activity of wheat germ

2.2.2.1 Determination of Minimum Inhibitory Concentration (MIC) of wheat germ extract

Each of 24 well plates was prepared to contain a giving volume of a media mixed with the extract due to the *law of volumes*, ranged from 2.5 to 0.039 mg/ml) were performed, beside the positive control (brain heart fusion) that contain media and the negative control (DMSO and media). Swabs of bacteria and fungus were prepared in advance and grown in broth media vials and number of Bactria was adjusted to contain approximately 1.5X10^8 CFU/mL, table (2.6) shows the McFarland standard preparation measured by spectrophotometer , 25 µl of each Bactria and yeast was taken and cultured on each well . The tested plates were incubated at 37 c for 18 hr. After 18 the inhibition of the growth can be detected .MIC was defined as the lowest sample concentration inhibit the growth (clear) and exhibited complete the inhibition of the growth *(*Turnidge *et al,. 2003)*.

2.2.2.2 Determination of Minimum Bactericidal Concentration Test

Pure cultures of bacteria were grown in broth media for overnight, then diluted in the test tubes growth-supporting broth (Muller Hinton Broth) to a concentration between 1 x 10^8 CFU/ml. Each dilution of the wheat germ extract was inoculated with an equal volume of the specified bacteria culture. Each tested bacteria was stated with a concentration of the stock solution of (2.5 mg/ml).The positive and negative controls were included for every test bacteria to demonstrate the adequate microbial growth over the course of the incubation period and medium sterility, respectively. An aliquot of the positive control was plated and used to establish a baseline concentration of the bacteria used. The tubes were incubated at 37 C for overnight (Cushnie *et al.,* 2007).

Table (2.6): McFarland Nephelometer Standards

McFarland Standard No.	0.5	1	2	3	4
Approx. cell density (1X10^8 CFU/mL)	1.5	3.0	6.0	9.0	12.0
% Transmittance*	74.3	55.6	35.6	26.4	21.5
Absorbance*	0.132	0.257	0.451	0.582	0.669

- Wave length in spectrophotometer used 625 nn.

2.2.3 Anti-cancer activity of wheat germ extracts experiment

2.2.3.1 Cell lines

a. Cell line culture for k562 cells myelogenous leukemia

The cells line culture was done at the cancer and stem cells research center, faculty of medicine, university of Jordan, The cell lines, originally obtained from the American Type Culture Collection (ATCC, USA).The K562 cells were maintained as an attached monolayer culture in the commercially defined RPMI 1640 medium (Gibco, USA), supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (HyClone, USA), 2 mM L-glutamine, 100 U/mL and 100 μg/mL penicillin– streptomycin (HyClone, USA), and 25 μM 4-(2 hydroxyethyl)-1- piperazineethanesulfonic acid (HEPES) (Lonza, USA),(Hus *et al.*,2012).

b. Cell line culture for fibroblast normal cells

The Fibroblast cells culture in the commercially defined DMEM-high glucose medium (Gibco, USA), supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) (HyClone, USA), 2 mM L-glutamine, 100 U/mL and 100 μg/mL penicillin– streptomycin (HyClone, USA) , The cells were grown on either 25 or 75 cm2 attached types, filter-cap culture flasks (NunClon, Denmark). The cells were then incubated at 37 °C in a 90% humidified atmosphere of 5% CO2.

c. Cell line culture for breast cancer (MCF-7)

The procedure of (**2.2.3.1.a**) was used expect (MCF-7) instead of (K562) cell line.

2.2.3.2 Methyl thiazolyl tetrazolium (MTT) proliferation assay for cell validity

The anti-proliferative effects of wheat germ (chloroform\methanol) and Methanol extract on MCF7 and Fibroblast cells were evaluated using the Cell Titer Non-Radioactive Cell Proliferation Assay Kit® (Promega, USA), according to manufacturer's instructions. This assay is a colorimetric test based on the reduction of 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), a yellow tetrazole, to a purple formazan, a process that occurs in the mitochondria of viable cells.

The cells were seeded onto 96-well plates (sigma, USA) at a concentration of 10×10^4 and 7×10^4cells/well and incubated for at least 18 hr. After incubation, dilutions of the drug ranging from 900 μg/ml to 1.8 μg/ml were added (100 μL/well). Each concentration was added in

triplicates, and every plate contained a control of cells in plain medium. The cells were then incubated at 37 °C for 72 hr. After incubation, the media were aspirated from the wells and replaced by fresh media (100 µL/well); 15 µL of the MTT dye solution was added to each well. The plates were incubated at 37 °C for 4 h, and then 100 µL of solubilisation/stop solution was added to each well. Optical density (OD) at 570 nm wavelength was recorded 1h later using a 96-well plate reader , K562 cells were seeded onto 96-well plates at a concentration of 4×10^4 cells/well were added (50 µL/well). Then 100 µL of solubilisation/stop solution was added to each well. Optical density (OD) at 570 nm wavelength was recorded 1h later using a 96-well plate reader (sprangers *et al*., 2003).

2.2.3.3 Data analysis

Results of the MTT cell proliferation assay were analyzed using the GraphPad PRISM®5.0 software (GraphPad Software, Inc.). The inhibitory concentration (IC_{50}) values, which are the drug concentration at which 50% of cells are viable, were calculated from the logarithmic trend line of the cytotoxicity graphs.

2.2.4 Treatment of atopic dermatitis by wheat germ extracts

2.2.4.1 Laboratory Animals

A total of male 15 BALB/c pathogenic free mice were employed in this study. They were supplied from (Jovac Vaccines Factory – Jordan). Their ranged from 2 – 4 months, and their weight between 30-35 grams.

Mice were kept for acclimatization before being used in the experiment. They were divided individually each mouse was housed in separated transparent plastic cage with stainless steel cover lid.

The animals were maintained at a temperature of 20 – 25˚C and they had free excess to food (standard pellets) and water throughout the experiments which were done in an animal isolator laminar with highly sterilization conditions.

2.2.4.2 Reagents used for atopic dermatitis induction

a-Isoflurane

b-Dermatophagoides *farinae* extract house dust mite (Der f) it was purchased from (Greer laboratories, Lenoir, NC) category number XPB81D3A2.5, (it cause and lead to allergic reactions in the skin (Barbara 1995), it was used and diluted in phosphate buffer saline.

C- Phosphate buffer saline (PBS) , was prepared by adding 800 mL of distilled water to dissolve all salts. The pH was adjusted to 7.4 with HCl. distilled water was added to a total volume of one liter. The resultant PBS had a final concentration of 10 mM PO_4^{3-}, 137 mM NaCl, and 2.7 mM KCl.

2.2.4.3 Wheat germ extract ointment preparation

Formulation of ointment was done by incorporating the active ingredients in the base by trituration using mortar and pestle. The prepared ointment was filled in tube and stored at room temperature. The ointment was composed from the followings

Emulsifying wax 25 g

White soft paraffin 30g

Liquid paraffin 25 g

Wheat germ extract 20 g

Emulsifying wax and liquid paraffin were melted by heat using Bunsen burner to melt them, white soft paraffin was added melting the tritateded during the experiment to produce yellow creamy ointment, then 20 g wheat germ extract were added to the mixture for a final concentration of 20% (w/w).

2.2.4.4 Stages of Inducing Atopic dramatis

Mice were anesthetized with Diethyl ether and was shaved (approximately a 3 x 3 cm area for application with an electric shaver) at the first day, and were anesthetize with Diethyl ether for the second day, the shaved back of the mice was adhered and detached skin with durable cloth tape this were repeated several times , 1x1 cm square of gauze pad (Johnson and Johnson) on the area of application were Placed , 10 g of *Dermatophagoides farinae* in 100µl , PBS onto the gauze pad were added the gauze and antigens onto the skin using a 2 x 2 cm piece of Tegaderm ' Transparent Dressing were Occluded completely covering the gauze. This prevents loss of the antigens due to ingestion or rubbing off, the entire system were kept in place using 8 x 2 cm Flexible Fabric Adhesive Bandages , Tightly wrapped the mouse with a resulting 8 x 1 cm bandage strip, covering the upper half of the underlying dressing .

2.2.4.5 Treatment for Atopic dermatitis

a- Mice given wheat germ extract

Wheat germ extract were applied directly on the skin and mice were not bandaged or not covered during 2 days of the treatment figure (2.3).

b- Mice given wheat germ ointment

Ointment with wheat germ extract were applied on broken skin on the skin.

c- Mice given blank ointment base (negative control)

The blank control ointment was prepared with ointment only without the extract and also applied directly on the skin topically.

d- Mice given betamethasone ointment (positive control)

Betamethasone Dipropionate Ointment 0.05 % contains betamethasone dipropionate USP, a synthetic adrenocortical steroid, for dermatologic use was applied directly to the skin as positive control.

2.2.4.6 Atopic dermatitis type scoring

The clinical conditions was evaluated According to the method described By Matsuoka (Matsuoka *et al*., 2003) briefly, severity of clinical signs was determined by the Sum of Two signs: dryness/ Scaling and Erosion/excoriation/hemorrhage which were graded by 0 (No Symptoms), 1 (mild), 2 (moderate) and 3 (severe).

Dermatitis scores evaluated at the beginning (3-week) doses of *Dermatophagoides farinae* extract were giving Every 3 days and watching for skin changes.

2.2.4.7 Radioallergosorbent test (RAST test)

Blood samples were taken from the mice, for each group of the experiment, blood samples were centrifuged 400 G, and serum were examined with RAST (wood *et al.*, 2007).

Table (2.8): Rast rating levels and comment for each range.

RAST rating	IgE level (IU/ml)	Comment
0	< 0.21	ABSENT OR UNDETECTABLE ALLERGEN SPECIFIC IgE
1	0.35 – 0.69	LOW LEVEL OF ALLERGEN SPECIFIC IgE
2	0.70 – 3.49	MODERATE LEVEL OF ALLERGEN SPECIFIC IgE
3	3.50 – 17.49	HIGH LEVEL OF ALLERGEN SPECIFIC IgE
4	17.50 – 49.99	VERY HIGH LEVEL OF ALLERGEN SPECIFIC IgE
5	50.00 – 100.00	VERY HIGH LEVEL OF ALLERGEN SPECIFIC IgE
6	> 100.00	EXTREMELY HIGH LEVEL OF ALLERGEN SPECIFIC IgE

3 Results and discussion

3.1.1 Yield of wheat germ extract

Chloroform methanol extraction resulted in 10.88g of fine yellow oil, while for methanol extraction the yielded 6.55 g very fine yellow oil.

3.1.2 GC-MS Analysis of wheat germ extractable results

GC-MS chromatogram analysis of the chloroform/methanol and methanol extracts of *wheat germ* showed 21 peaks which indicating the presence of 21 phytochemical constituents. On comparison of the constituents mass spectrum with the NIST library, the 21 phytocompounds were characterized and identified as shown in (table 3.1), (table 3.2) .The mass spectra of all the phytochemicals identified in the whole plant chloroform and chloroform/methanol extract of *wheat germ* presented in (appendix.1),(appendix 2). Of the 21 compounds identified, the most prevailing compounds were linoleic acid (38.09%), oleic acid compound (23.11%) and Hexadecanoic acid (13.10%). Among the compounds, two compounds were reported to have anticancer activity, and antimicrobial activity, anti-eczema tic activity, oleic acid and linoleic acid (Duke, 2009).

Table (3.1): Identification of GC-MS amendable compounds and their intensities for chloroform methanol extract.

Peak No.	RT, min	Intensity	Percentage (%)	Name of compound
1	3.32	4657	1.14	Glycerin
2	6.3	1860	0.45	5Hydrxoymethylfurfural
3	7.16	675	0.16	m-Ditertbutylbenzene
4	7.97	338	0.08	2,4,6,8-Tetramethyl-1-undecene
5	8.09	325	0.08	2,4,6,8-Tetramethyl-1-undecene
6	8.2	289	0.07	2,4,6,8-Tetramethyl-1-undecene
7	9.73	1743	0.43	Butoxyacetic acid
8	10.8	653	0.16	3',5'-Dimethoxyacetophenone
9	13.2	318	0.08	Tetradecanoic acid
10	15.36	53645	13.10	Hexadecanoic acid
11	16.83	12202	2.98	Linoleic acid
12	17.0	155974	38.09	Linoleic acid
13	17.05	94631	23.11	Oleic acid
14	17.22	11318	2.76	Octadecanoic acid
15	19.96	6596	1.61	Glycerol beta-palmitate
16	21.29	13815	3.37	Beta-Monolinolein
17	24.5	5511	1.35	Vitamin E
18	24.92	8073	1.97	5-Pentadecylresorcinol
19	25.39	8141	1.99	Campesterol
20	26.16	21144	5.16	Beta-Sitosterol
21	26.65	7564	1.85	5-Pentadecylresorcinol

Table (3.2): Identification of GC-MS amendable compounds and their intensities for methanol extract.

Peak No.	RT, min	Intensity	Percentage (%)	Name of compound
1	3.32	36500	9.84	Glycerin
2	6.3	1795	0.48	5Hydrxoymethylfurfural
3	7.16	668	0.18	m-Ditertbutylbenzene
4	7.97	334	0.09	2,4,6,8-Tetramethyl-1-undecene
5	8.09	356	0.10	2,4,6,8-Tetramethyl-1-undecene
6	8.2	320	0.09	2,4,6,8-Tetramethyl-1-undecene
7	9.73	1795	0.48	Butoxyacetic acid
8	10.8	512	0.14	3',5'-Dimethoxyacetophenone
9	13.2	202	0.05	Tetradecanoic acid
10	15.36	45371	12.23	Hexadecanoic acid
11	16.83	N/A	0.00	Linoleic acid
12	17.0	133242	35.93	Linoleic acid
13	17.05	80403	21.68	Oleic acid
14	17.22	9457	2.55	Octadecanoic acid
15	19.96	5365	1.45	Glycerol beta-palmitate
16	21.29	11548	3.11	Beta-Monolinolein
17	24.5	4706	1.27	Vitamin E
18	24.92	6874	1.85	5-Pentadecylresorcinol
19	25.39	6954	1.88	Campesterol
20	26.16	18045	4.87	Beta-Sitosterol
21	26.65	6429	1.73	5-Pentadecylresorcinol

3.2 Antimicrobial activity of wheat germ extracts

3.2.1 Minimum inhibitory concentration (MIC) of wheat germ extracts

Results of minimum inhibitory concentration (MIC) showed that Chloroform/methanol extraction of wheat germ had antibacterial activity, much higher than the methanol extraction with MIC values ranging from 2.5 to 0.165 mg/ml depending on the species of bacteria and yeast table (3.3).The tested extracts showed different levels of antimicrobial activity

depending on tested species as shown in table (3.3).The (MIC) for the wheat germ extract was the lowest for the *Enterococcus faecalis* figure (3.1) while there was no effect for the extract against the *Micrococcus luteus* for both methanol and methanol chloroform extraction , it was the first time reporting (MIC) for a yeast species for the *Candida albicans* , *C. tropicalis* species with a (MIC)of 2.5 mg/ml figure (3.2) , due the results that shows the high antibacterial , antifungal activity of the wheat germ extract might refer to the high levels of linoleic acid and oleic acid in the extract . (Dilika *et al.,* 2000) a study mentioned that the antibacterial activity-guided fractionation of the dichloromethane extract of leaves of Helichrysum pedunculatum resulted in the isolation of linoleic and oleic acids. Linoleic acid and oleic inhibited the growth of all the Gram-positive bacterial species tested, for the gram negative strains MIC vary (0.1,1.0 mg/ml) and fungal strain were tested it's still unclear what the responsible for the activity was against these strains.

3.2.2 Minimum bactericidal concentration (MBC) of wheat germ extract

Minimum bactericidal concentration of the wheat germ extracts was determined against bacteria strains had the highest antimicrobial activities shown no growth .The results were the same with minimum inhibitory concentrations for chloroform methanol and methanol extracts, for MIC and MBC table (3.2).

Table (3.3): Strains of microorganism used and MIC and MBC for extracts.

Strains of Microbial strains Tested	**Chloroform methanol extract**		**Methanol extraction**	
	MIC mg/mL	MBC	MIC mg/mL	MBC
pseudomonas aeruginosa ATCC 9027	1.25	2.5	-	-
Serratia marcescens ATCC13880	2.5	2.5	-	-
Micrococcus luteus ATCC 9341	-	-	-	-
Klebsiella pneumonia ATCC 1705	1.25	2.5	2.5	2.5
Enterococcus faecalis ATCC 29212	0.165	0.165	1.25	1.25
Salmonella typhimurium ATCC2054	0.625	0.625	2.5	2.5
Staphylococcus epidermidis ATCC 12228	2.5	2.5	-	-
Staphylococcus Aureus ATCC1927	1.25	1.25	-	-
Candida abanis ATCC 90028	2.5			
Candida tropicalis	2.5			
Bacillus cereus ATCC 1014	0.625	0.625	2.5	2.5
Escherichia coli ATCC 2593	1.25	1.25	-	-

Figure (3.1): MIC of wheat germ extract against for *Enterococcus faecalis.*

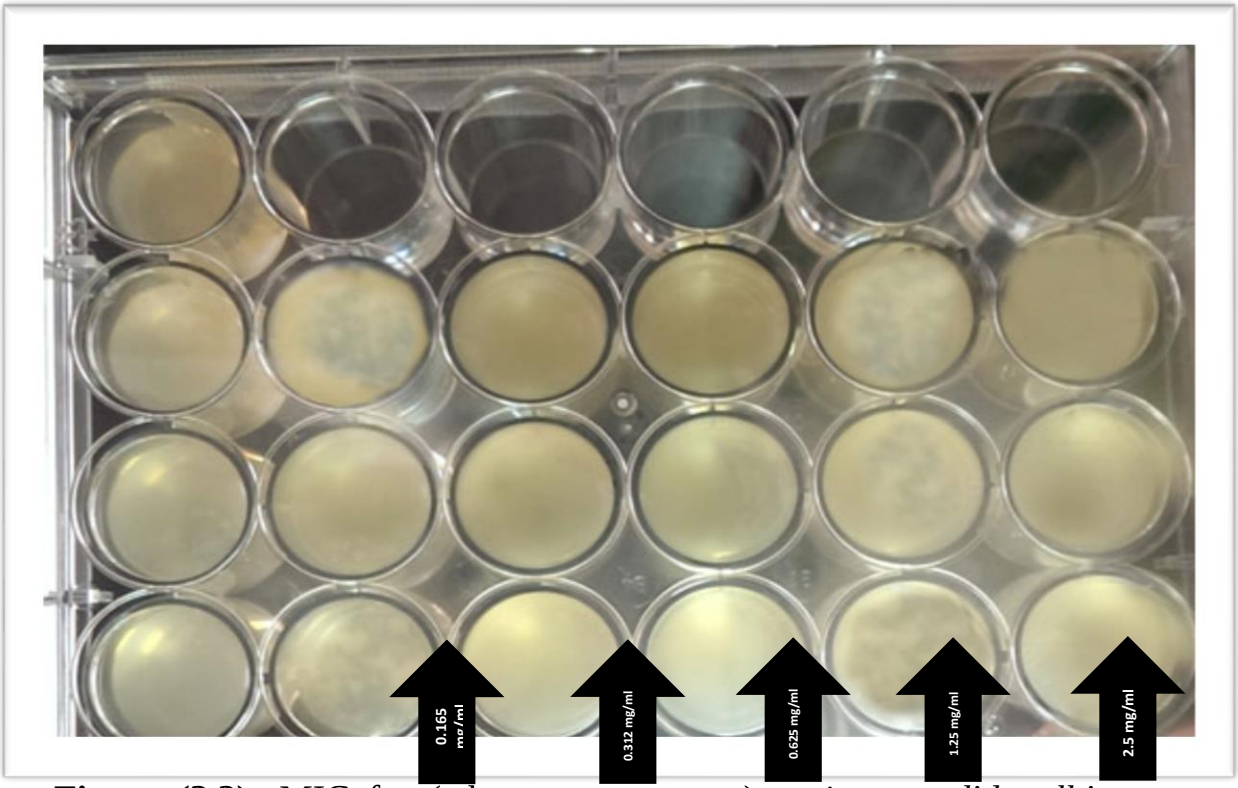


Figure (3.2): MIC for (wheat germ extract) against *candida albicans* ATCC 90028.

3.3 Anti-cancer activity of wheat germ extracts on cell line

3.3.1 Cytotoxic effect of methanol/chloroform extracts on (K562) cell line and MCF-7 cell line and fibroblast cell line

The antitumor activity of the synthesized compounds were characterized by conducting cell viability assay using tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Cultures of the breast cancer cell lines MCF-7 and Leukemic cell line K562 were treated with concentrations start from 900 to 14.06 µg/ml and the results are shown in table (3.4). In the MCF-7 screening test, chloroform/methanol extract showed a potential anti–MCF7 activity at 900 µg/ml concentrate, and at 450 µg/ml figure (3.3). In K562 screening test it showed anti-K562 activity for extraction at 900, 450, 225 and 113 µg/ml concentrate, wheat germ compounds were able to reduce the proliferation to less than 50% for the k562 cells, which indicate that those compounds may have a potential anti K562 activity and the IC_{50} determined values for the potential compounds against K562 was 189.6 µg/ml, (figure 3.3). Cultures of the breast cancer cell line MCF-7 were treated and resultant IC_{50}s were less in activity than the K562 cells 473.2 µg/ml, figure (3.5). From the structure-activity relationships point of view, the nature of wheat germ extract, seems to play a critical role in determining the anti-cancer activity, when fibroblast cells were treated also with wheat germ extract no activity shown for the extract.

3.3.2 Effect of methanol extracts on K562 cells and MCF-7 cell line

The MTT assay results of this extraction method declared that it was selective for the K562 cells on high dosage of wheat germ extract but less toxic for the K562 cells with IC_{50} 242.2 µg/ml, (figure 3.4), however this assay gave week compared with activity compares with the

chloroform methanol extraction on the other hand there was no activity for the extract on MCf-7 cell line and that may refer to the low percentage of oleic acid produced compared to the methanol/chloroform extract.

Due to the high presence of Oleic acid in wheat germ extract, (table 3.3) (Carrillo *et al.*, 2012) found that presence of Oleic acid of could suppress the over-expression of HER2 (erbB-2), a well-characterized oncogene which plays a key role in the etiology, invasive progression and metastasis in several human cancers. In addition, oleic acid could play a role in intracellular calcium signaling pathways linked to the proliferation event. Regarding cell death, oleic acid has been shown to induce apoptosis in carcinoma cells. The mechanisms behind the apoptotic event induced by oleic acid could be related to an increase in intracellular ROS production or caspase 3 activity.

3.3.3 Effect of methanol/chloroform and methanol extracts on fibroblast cell line

There were no cytotoxicity of the wheat germ extracts for both methanol and chloroform/methanol extracts, after treating the cells with the therapeutic dosage that worked on the K562 cells and MCF-7 cells were not affected and gave an IC_{50} of 450 µg/ml very close to the positive and negative control that mean wheat germ does not have any potential activity on normal cells.

Table (3.4): The IC_{50} values for the 2 tested wheat germ extracts against MCF-7 and K562 cell lines.

Extraction method	**IC_{50} µg/ml MCF-7**	**IC_{50} µg/ml K562**	**IC_{50} µg/ml Fibroblast**
Methanol/chloroform	473.2	189.6	No activity
Methanol	No activity	271.9	No activity

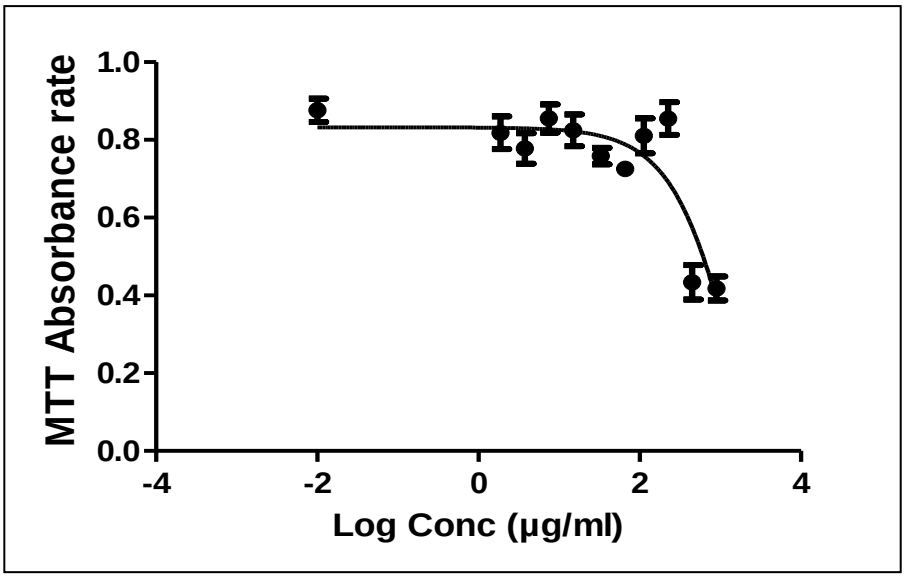


Figure (3.3): The dose response curve showing the IC_{50} value of the wheat germ and the effect of different concentrations of antagonist on reversing agonist activity, wheat germ was selective to the k562 and gives an IC_{50} of 189.6 (µg/ml).

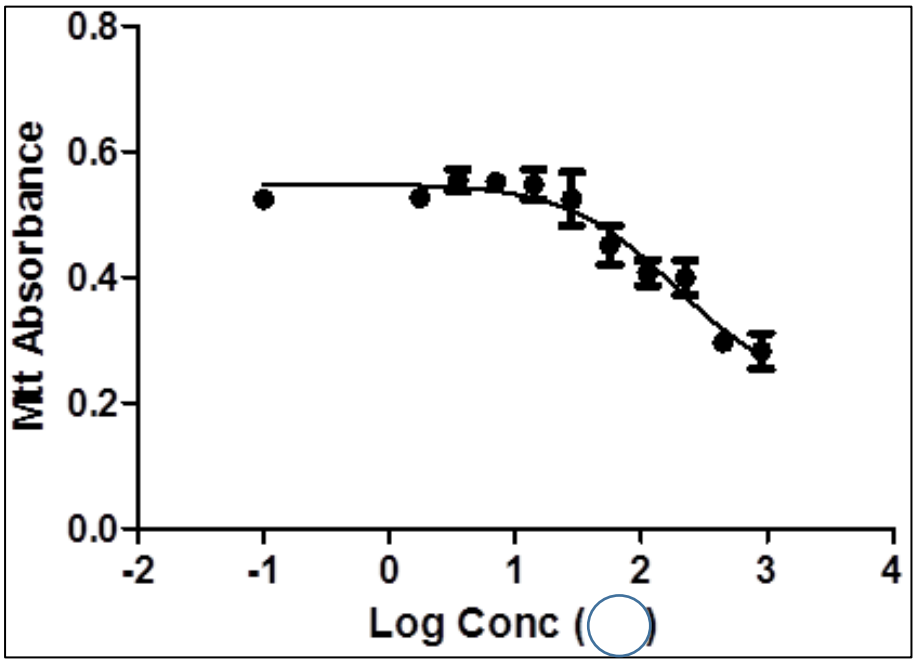


Figure (3.4): The dose response curve showing that the methanolic extract of the wheat germ on K562 cell had less activity than the chloroform/methanol extract given IC_{50} 271.9 µg/ml.

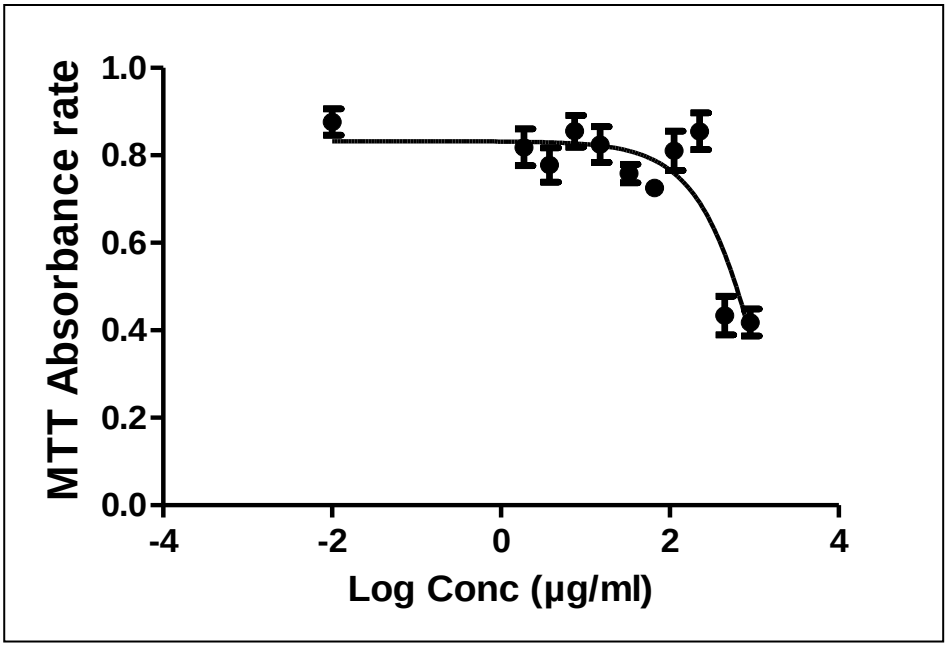


Figure (3.5) : The dose response curve showing the IC_{50} value of the wheat germ and the effect of different concentrations of antagonist on reversing agonist activity, wheat germ was selective to the MCF-7 and gives an IC_{50} of 473.2 (µg/ml).

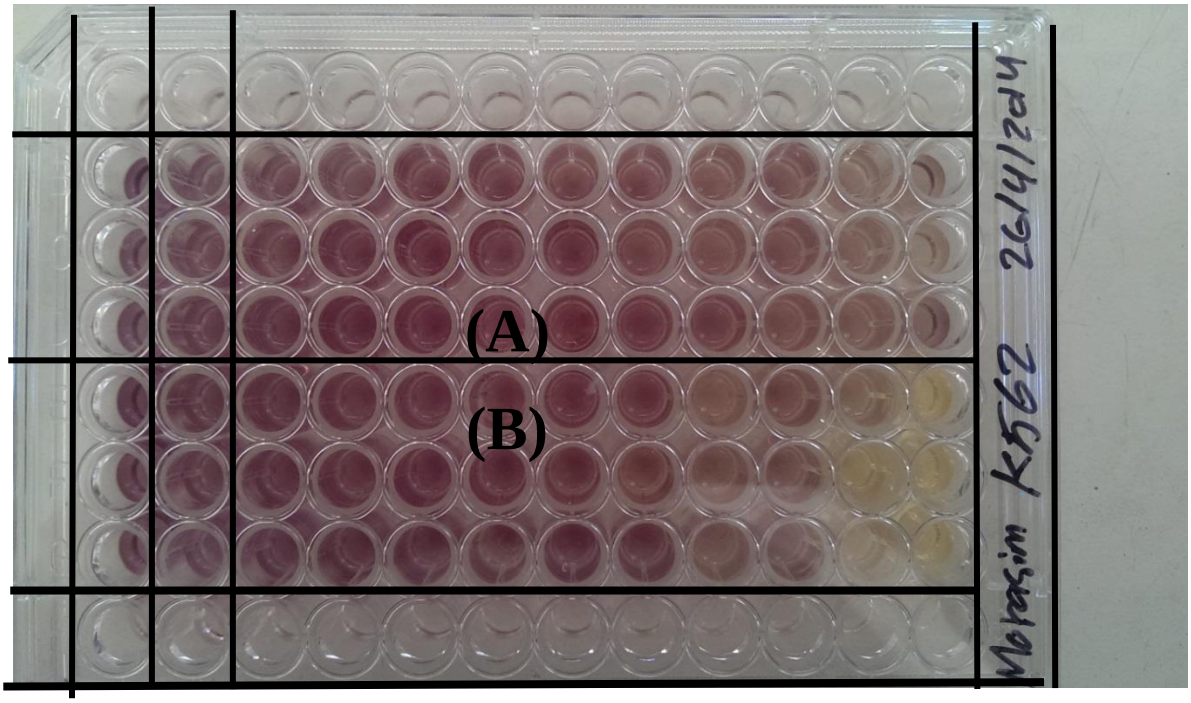


Figure (3.6): 96 plate, after an MTT assay, increasing amounts of cells resulted in increased purple coloring (A): Methanol extract were showing less

toxicity to the cells, (B): Chloroform Methanol extract shows more toxicity and ability to kill cancer cells.

3.4 Results of Inducing Atopic Dermatitis

Atopic dermatitis were induced in seven mice and the symptoms were ranging between sever, moderate, and mild symptoms, by repetitive epicutaneous application treatment of *dermatophagoides farinae* extract allergen dermatitis –prone were induced and results were histologically and immunologically similar to human atopic dermatitis as in (kawakami *et al ,. 2007*), and those mice were examined by veterinary doctor Shadi Amarin specialists in the research center of animal house of jovac vaccines factory / Jordan.

3.4.1 Evaluation of atopic dermatitis

The clinical conditions was evaluated according to the method described By Matsuoka (Matsuoka *et al.*, 2003) briefly, severity of clinical signs was determined by the Sum of Two signs: dryness/ Scaling and Erosion/excoriation/hemorrhage which were graded by 0 (No Symptoms), 1 (mild), 2 (moderate) and 3 (severe).Dermatitis scores evaluated at the beginning (3-week) and at theEnd (6-week).

3.4.2 Effect of wheat germ on atopic dermatitis

a- Wheat germ extract treatment results

Mice given a treatment of wheat germ topically were applied to the skin of a model mice with a severe symptoms, shown a healing after two days with no symptoms of cracks on the skin and no redness and the scratching behavior was totally disappeared skin shown at figure (3.7),

(3.8) blood samples were taken from the mice and the IgE levels shows absent or undetectable allergen specific.

b- Effect of wheat germ ointment 20% treatment

Ointment was used as a topical application on the skin of mice, after two days. Results showed that the outcome ointment extract was relatively good, the symptoms were responded by change the color of the skin and the redness was disappeared just a small part of the skin, the ointment were given to mice with a moderate symptoms of Atopic dermatitis, after given the ointment blood samples taken and mice were sacrificed, the results of IgE specific to the allergen were very low 0.62 , 20% of the extract in the ointment were used to find the minimum concentration that cure for the atopic dermatitis figure (3.9).

c- Blank ointment base

Blank ointment base were applied topically to the skin for Atopic dermatitis mice model of a moderate symptoms, no changes after two days

happened to the skin Erosion and excoriation was still presence on the skin IgE levels specific to the allergen was ≥ 0.80, this treatment was taken as a control for the experiment, the result shown the ointment material was not effective for the skin figure (3.10).

d- Betamethasone ointment 0.05

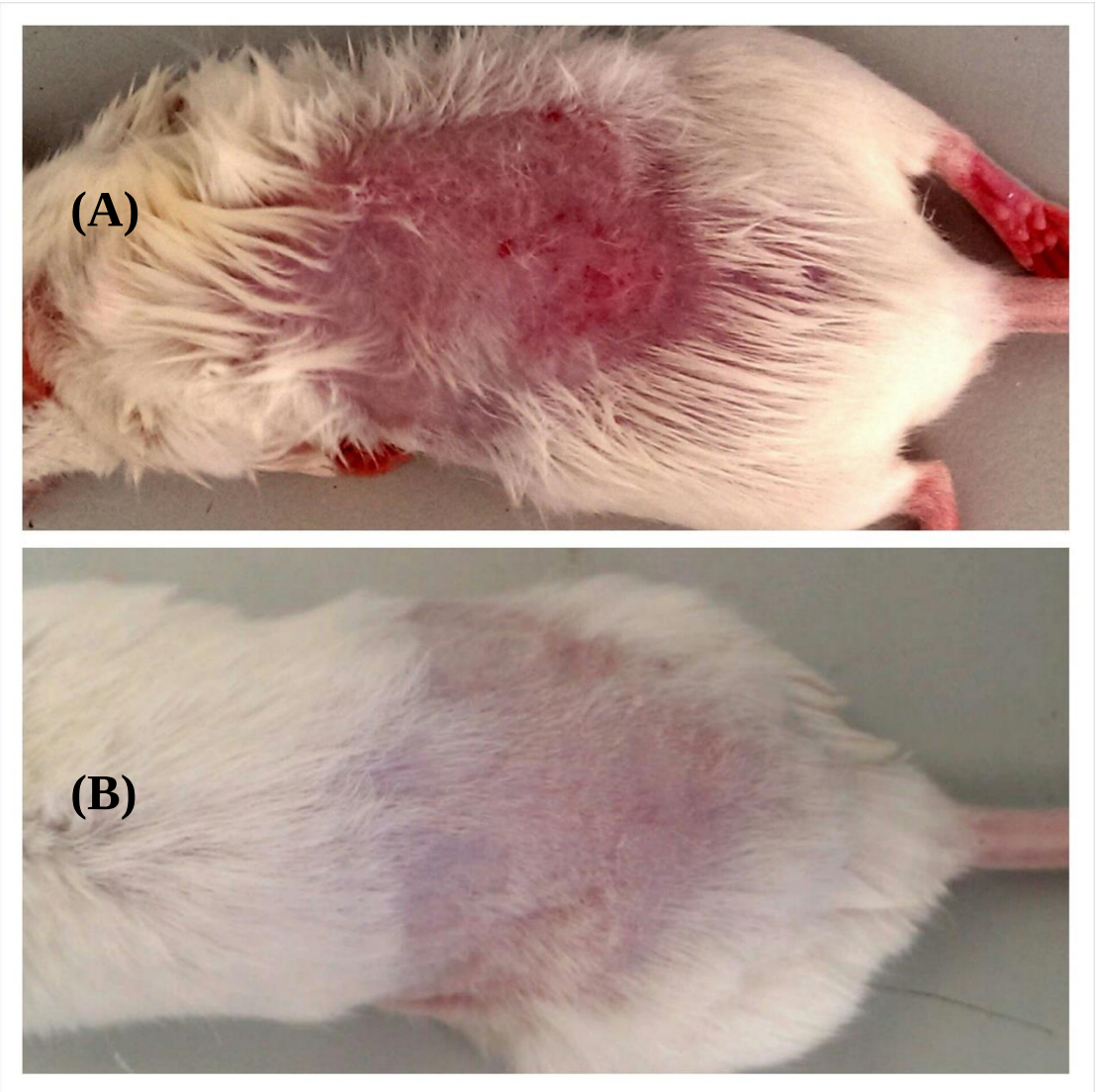
Betamethasone used and applied directly to the skin, for a mice with a moderate symptoms after two days the results shown that no symptoms of atopic dermatitis shown this experiment were taken as a control for the experiment with a levels of IgE ≤ 0.21, figure (3.11).

No	Mice AD score	Number of mice	Symptoms
1	No symptoms	9	No symptoms shown at the skin
2	Mild	0	Dryness and scaling
3	Sever	2	Excoriation and hemorrhage
4	Moderate	5	Erosion and excoriation

Table (3.5): number of mice and their Atopic dermatitis score and note for the Signs.

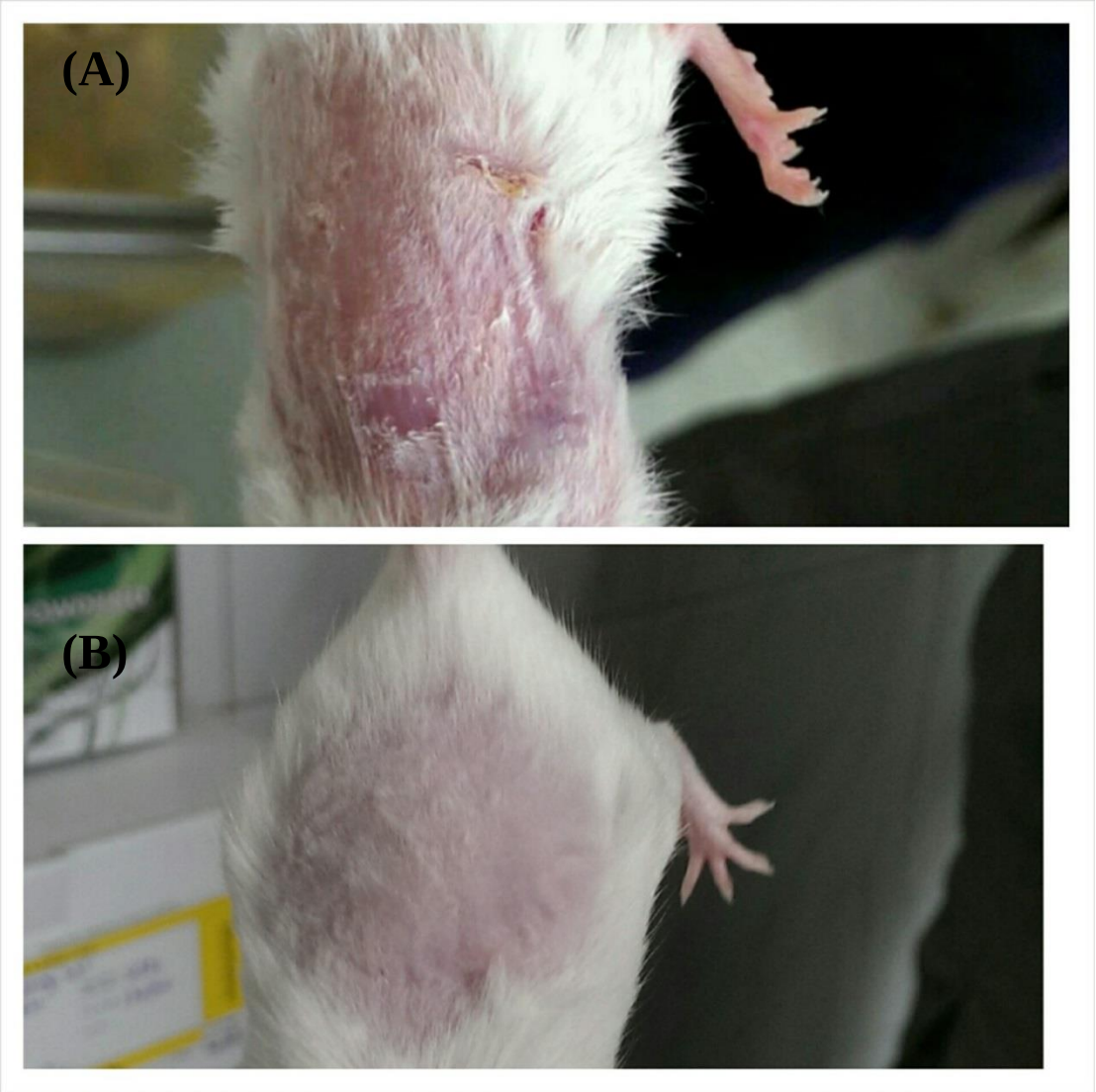
Table (3.6): Mice Atopic dermatitis score and IgE levels after treatment.

No	Mice AD score	Treatment with	Number of mice treated	IgE levels	Comment
1	sever	Wheat germ extract	1	< 0.21	Absent or undetectable allergen specific IgE
2	Moderate	Wheat germ extract	1	< 0.21	Absent or undetectable allergen specific IgE
3	Moderate	Ointment wheat germ 20%	2	0.62	Low levels of allergen specific IgE
4	sever	Ointment base	1	0.80	Moderate level of allergen specific IgE



Figure(3.7) : mice giving treatment of wheat germ oil applied directly to the skin (A) mice having a score of 3 sever Atopic Dermatitis , (B)

mice after giving a treatment of wheat germ oil for 2 days IgE to house dust mite <0.21 (IU/ml).



Figure(3.8) : mice giving treatment of wheat germ oil applied directly to the skin (A) mice having a score of 4 moderate Atopic

Dermatitis , (B) mice after given a treatment of wheat germ oil for 2 days IgE to house dust mite <0.21 (IU/ml).

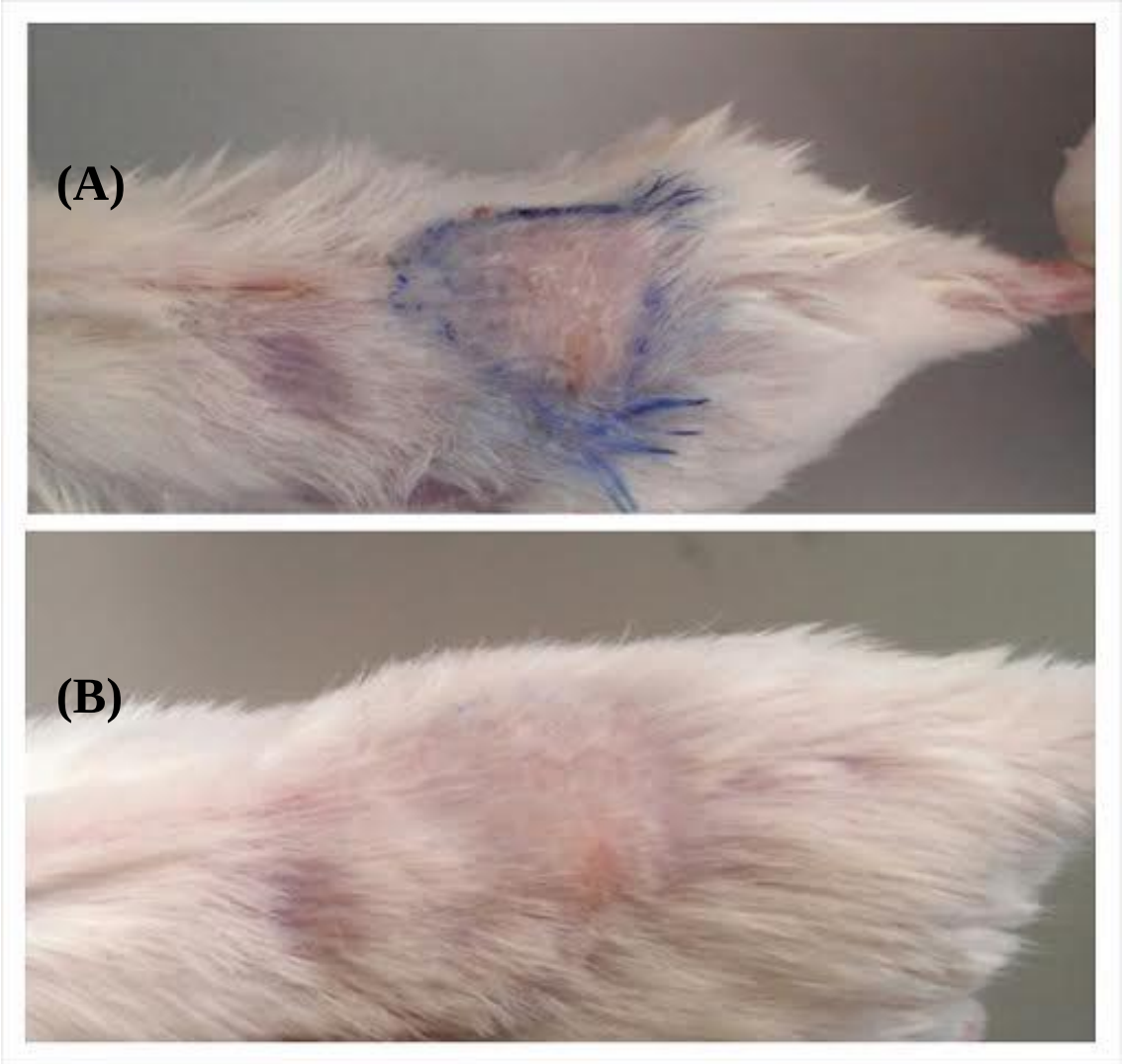


Figure (3.9) :(A) mice without treatment score eczema 4 moderate, (B): mice after 2 days given ointment 20% wheat germ oil score of IgE 0.62 (IU/ml) spore of atopic dermatitis on side presence.

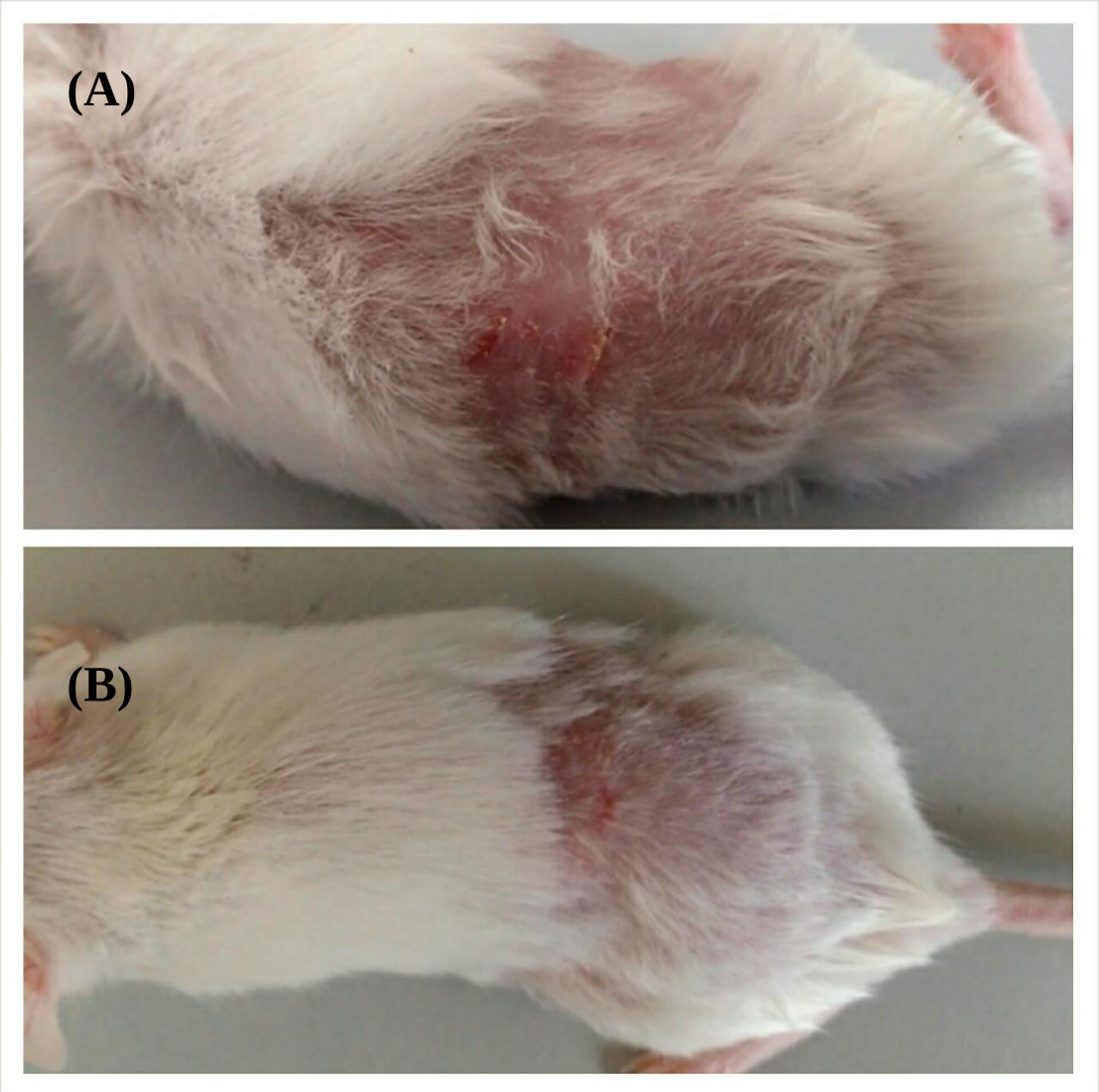


Figure (3.10): control mice showing a score of 3 sever Atopic dermatitis treated with ointment base **(A):** mice before adding ointment

base to skin, **(B):** mice giving ointment base treatment for 2 days showing a score of atopic dermatitis IgE was 0.80(IU/ml).

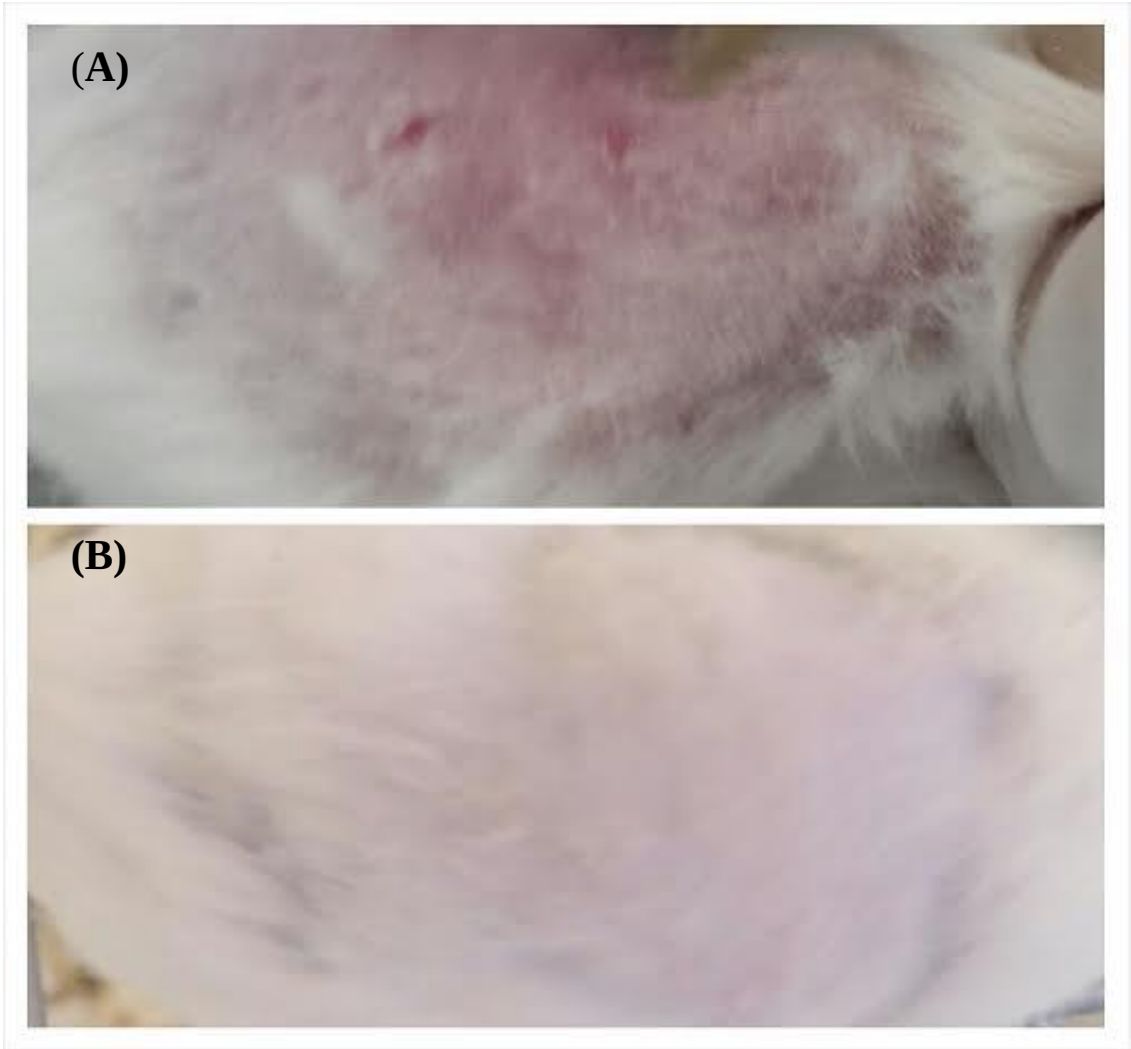


Figure (3.11): (A): control mice without treatment score Atopic dermatitis was 4 moderate, (B): mice after given betamethasone ointment *0.05%* 2 days giving ointment score of IgE < 0.21 (as a positive control).

Due to the high presence of linoleic acid in the wheat germ extract a percentage of 40.1% it might refer to the fast healing process for the atopic dermatitis.

Alterations in linoleic acid metabolism have been demonstrated in atopic conditions such as eczema (Geppert J *et al.*, 2008) Conversion of linoleic acid to gamma linoleic acid is inhibited in individuals with atopic dermatitis. During the past two decades several studies have reported mixed results on the use of Gama linoleic acid -containing oils, particularly EPO, for atopy (Miyake, 2009) these studies reveal subtle improvements, such as decreased inflammation and itching.

Topical essential fatty acids have been shown to improve the structure and function of cell membranes and improve skin barrier function. Improving skin barrier function reduces trans-epidermal water loss, leaving the skin more hydrated, moisturized and protected (Horrobin and Stewart ,1991) While other moisturizers may improve skin dryness, roughness and water loss, they are often temporary and do not biologically strengthen the integrity of the skin barrier as essential fatty acids do.

Chapter Four
Conclusions and Recommendations

4.1. Conclusions

1- Different classes of compounds were detected in wheat germ extracted by methanol and methanol/chloroform including polyunsaturated fatty acids.

2-Chloroform / methanol extract of wheat germ gave the highest anti-microbial activity against both gram negative and positive bacterial strains as well as yeast strains used in the experiments.

3- Chloroform / methanol extract of wheat germ showed toxicity and selectivity for the K562 cancer cells and MCF-7 tumor cells, on the highest dosage of wheat germ extract and showed no toxicity to the normal cell line which is a very promising result that indicate the using of the extract as a therapeutic drug or as a supplement for the patients with Lukima and breast cancer without any concerns of high dosage.

4-Wheat germ extract can used as a topical ointment for the patients of Atopic dermatitis (eczema) without using harm and hazard materials that and many toxic drugs used as a cure like cortisone derivatives.

4.2. Recommendations

1- Qualitative and quantitative study of different active compounds present in wheat germ extract is need especially studying the activity of specific compounds. (HPLC) analysis

2- Further studies on the effects of wheat germ extract *on* skin infections and disease because the GC-MS analysis showed many fatty acids and components that might have an high bioactivity for skin treatments.

3- More mice models needed for experiments of atopic dermatitis must improve by using another materials that were prevented in Iraq and Jordan especially *staphylococcal enterotoxin* b (seb), which have the key role in inducing atopic dermatitis in the mice model and the lack of this toxin prevent this study to complete the experiment of the inducing atopic dermatitis mice model and which made the experiment with a low samples of mice models.

References

- **Abiramasundari.P**.; Priya .V.; Jeyanthi.G.P.; and Gayathri Devi. S. (2011) Evaluation of the Antibacterial activity of Cocculus hirsutus, Hygeia.J.D.Med, 3(2): 26-31.

- **Adom, K.;** Sorrells, M .; and Liu, R. (2005). Phytochemicals and antioxidant activity of milled fractions of different wheat varieties. J. Agric. Food Chem, 53**,** 2297-2306.

- **Ahmedin**, Rebeca Sengal.; Elazabtha ward.; tommy bishob. (2007). Cancer Statistics, CA.Cncer J.Clin. 57:43-66.

- **Alan L**. (2008). Natural products in drug discovery, Drug Discov Today, 13, 19/20.

- **Alessandra** , P.; Antonella , R.; Danilo , F.; Porcedda, S .; Dessì, M. A.; and Bruno , M. (2009). Extraction of Oil from Wheat Germ by Supercritical CO2. molecules, 14:1420-3049.

- **American Cancer Society**. Cancer Facts &2013. Atlanta, GA: American Cancer Society; 2013.

- **Anderson**, J. W.; Hanna, T. J.; Peng, X.; Kryscio, R. J. (2000). Whole grain foods and heart disease risk. ACN**,** 19, (3):291S-299S.

- **Andreassi** M .; Forleo P .; Di Lorio AL. (1997). Efficacy of gamma-linolenic acid in the treatment of patients with atopic dermatitis. J Int Med Res; 25:266-274.

- **Andrews**, J. M. (2001). "Determination of minimum inhibitory concentrations". J. Antimicrob. Chemother. 48, (1); 5-15.

- **Anita Koltay** .; Norelle L. Daly .; Kirk R. Gustafson .; David J. Craik.(2005) "Structure of circulin B and implications for antimicrobial activity of the cyclotides," INT J PEPT RES THER, 11, (1); 99–106.

- **Azzi A** .; Stocker A. (2000). Vitamin E: non-antioxidant roles. Prog LipidRes; 39:231-55.

References

- **Bakshi A**.; Mukherjee D.; Bakshi A. (2003).Gamma-linolenic acid therapy of human gliomas. Nutrition; 19:305-309.

- **Bartnik**, **M**.; Jakubczyk, T. (1989). Chemical composition and the nutritive value of wheat bran World Rev Nutr Diet**,** (60), 92-131.

- **Bartsch H.;** Nair J.; Owen RW. (2002)." Exocyclic DNA adducts as oxidative stress markers in colon carcinogenesis: potential role of lipid peroxidation, dietary fat and antioxidants". Biol Chem.; 383(6):915-21.

- **Beattie**, **P.E**.; Finlan, L.E.; Kernohan, N.M.; Thomson, G.; Hupp, T.R.; Ibbotson, S.H. (2005). "The effect of ultraviolet (UV) A1, UVB and solar-simulated radiation on p53 activation and p21Waf1/Cip1". Br. j dermatol, 152 (5) , 1001-1008.

- **Belch JJ**.; Hill A (2000). "Evening primrose oil and borage oil in rheumatologic conditions". Am. J. Clin. Nutr. 71 (1): 352S–6S.

- **Belk JP**.; He F.; Jacobson A. (1999) Overexpression of truncated Nmd3p inhibits protein synthesis in yeast. *RNA 5* (8):1055-70.

- **Benayache. S**.; Benayache. F.; and Benyahia. S (2001). Leaf Oils of some Eucalyptus Species Growing in Algeria. J. Essent. Oil Res., 13:210-213.

- **Benhassaini. H**.; and enabderrahmane. K and Chi. K (2003). Contribution to the assessment of the antiseptic activity of essential oils and oleoresin of Pistacia tial Atlas on some microbial sources: Candida albicans (ATC 20027), Candida albicans (ATCC 20032) and s accharomyces cerevisiae: ethno pharmacology, (30): 38-46.

- **Benjilali B**.; Tantaoui-Elaraki A.;Ismaïli-Alaoui M.; Ayadi A. (1986). Méthode d'études des propriétés antiseptiques des huiles essntiellespar contact direct en milieu gélosé. Plant Méd. Phytotherapy, 20:155-167.

- **Berke, R**.; Singh, A.; Guralnick, M (2012). "Atopic dermatitis: an overview". Am.Fam.phys. 86 (1): 35–42.

- **Berridge MV**.; Herst PM .; and Tan AS. (2008). Tetrazolium dyes as tools in cell biology: new insights into their cellular reduction. Biotechnol Annu Rev; 129 (1): 14–30.

References

- **Boscoboinik D**.; Szewczyk A.; Hensey C, Azzi A.(1991). Inhibition of cell proliferation by α-tocopherol. Role of protein kinase. J Biol Chem. 266:6188-94.

- Uptake and RNA ribose formation but increases fatty acid synthesis in MIA pancreatic adenocarcinoma cells". Pancreas 23 (2): 141–7.

- **Brigelius-Flohe R**.; Traber MG. (1999).Vitamin E: Function and metabolism. FASEB J. 13:1145–55.

- **Carrasco, F**.(2009)."Ingredientes Cosméticos".Diccionario de Ingredientes (4th ed.) France. 428.

- **Carrillo. C**.; del MM .; Cavia,R S .; and Alonso Torre.2012. "Antitumor effect of oleic acid; mechanisms of action. A review." Nutr Hosp 27 (5): 1860-1865.

- **Carsten Flohr** .; Michael Perkin .; Kirsty Logan .; Tom Marrs .; Suzana Radulovic .; Linda E Campbell.; Stephanie F MacCallum .; W H Irwin McLean and Gideon Lack. (2014)." Atopic Dermatitis and Disease Severity Are the Main Risk Factors for Food Sensitization in Exclusively Breastfed Infants" j invest dermmatol. 134(2):345-50.

Chanda. S and Rakholiya. K .(2011). Combination therapy: Synergism between natural plant extracts and antibiotics against infectious diseases. Science against microbial pathogens. Communicating current research and technological advances A. Méndez-Vilas (Ed.). 2:693-1348.

- **Claude P** .; Selitrennikoff .(2001). "Antifungal Proteins," Appl. Environ. Microbiol, 67, (7):2883–2894.

- **Coultate, T**. Food, the chemistry of its components; The Royal Society of Chemistry: London,UK, 1989; Chapter 4.

- **Colvin G. A**., Elfenbein G. J. (2003). "The latest treatment advances for acute myelogenous leukemia". Med. Health R I. 86 (8): 243–6.

- **Craik DJ** .; Daly NL .; Mulvenna J.; Plan MR.; Trabi M. (2009). Discovery, structure and biological activities of cyclotides ," Adv. Drug Deliv. Rev. 61, (11):918–930.

References

- **Cushnie TPT**, Hamilton VES, Chapman DG, Taylor PW, Lamb AJ (2007). "Aggregation of Staphylococcus aureus following treatment with the antibacterial flavonol galangin". J Appl Microbiol. 103 (5):1562–1567.

- **Daly NL**., Koltay A., Gustafson KR., Boyd MR.,Casas-Finet JR., Craik DJ. (1999). "Solution structure by NMR of circulin A: a macrocyclic knotted peptide having anti-HIV activity," j.mol.biol, 285, (1):333–345.

- **Drago, S**.; El Asmar, R.; Di Pierro, M.; Grazia Clemente, M.; Tripathi, A.; Sapone, A. (2008).zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines .Scand J Gastroenterol, *41*(4), 408-419.

- **Demidov LV**.;Manziuk LV.; Kharkevitch GY.(2008).Adjuvant fermented wheat germ extract (Avemar) nutraceutical improves survival of high-risk skin melanoma patients: a randomized, pilot, phase II clinical study with a 7-year follow-up. *Cancer Biother Radiopharm.*; 23(4):477-482.

- **Dilika F**.; Bremner PD .; Meyer JJ. (2000). Antibacterial activity of linoleic and oleic acids isolated from Helichrysum pedunculatum: a plant used during circumcision rites. Elsevier, 71(4):450–452.

- **Dondlinger, P. T**. (1908). The consumption of wheat. In The Book of Wheat Dondlinger, P. T., Ed. U.S. Dept. of Agriculture: 1908; 283-302.

- **Dunford, N.T**.; Zhang, M.Q.(2003). Pressurized solvent extraction of wheat germ oil. Food Res. Int., 36: 905-909.

- **DuPont, F. M**.; Chan, R .; Lopez, R .; and Vensel, W. H. (2005). Sequential extraction and quantitative recovery of gliadins, glutenins, and other proteins from small samples of wheat flour. J Agric Food Chem, *53*(5), 1575-1584.

- **Duke, J. A.** 2009. The Green Pharmacy Guide to Healing Foods. Emmaus, Pennsylvania: Rodale Press.

Ehrenfeld M.; Blank M.;Shoenfeld Y.(2001). AVEMAR (a new benzoquinone-containing natural product) administration interferes with the Th2 response in experimental SLE and promotes amelioration of the disease. *Lupus*; 10(9):622-627.

References

- **Epand RM,** Vogel HJ. (1999). "Diversity of antimicrobial peptides and their mechanisms of action," Biochimica et Biophysica Acta, 1462, (1-2): 11–28.

- **Fajka-Boja R** .; Hidvégi M.; Shoenfeld Y.(2002). "Fermented wheat germ extract induces apoptosis and downregulation of major histocompatibility complex class I proteins in tumor T and B cell lines". Int. J. Oncol. 20 (3): 563–70.

- **Ferguson, L. R** .; Harris, P. J.(1999). Protection against cancer by wheat bran: role of dietary fibreand phytochemicals. Eur J Cancer Prev**,** 8, (1):17-25.

- **Fernandez de Caleya R** .; Gonzalez-Pascual B .;García-Olmedo F, Carbonero P: (1972). "Susceptibility of phytopathogenic bacteria to wheat purothionins in vitro," Applied microbiology, 23, (5), 998–1000.

- **Fruits and vegetables**. J Am Coll Nutr **2000,** 19, (3 Suppl), 312S-319S.

- **Fuiano, N.;** Incorvaia, C. (2012). "Dissecting the causes of atopic dermatitis in children: less foods, more mites." Allergol.Int 61 (2): 231–43.

- **Gamma Linolenic Acid**. American Cancer Society. 13 May 2010. Retrieved August 2013.

- **Garami M**.; Schuler D.; Babosa M.;Borgulya G.; Hauser P.;Müller J,; Paksy A, Szabó E.;Hidvégi M .; Fekete G.J.(2004). Fermented wheat germ extract reduces chemotherapy-induced febrile neutropenia in pediatric cancer patients. Pediatr Hematol Oncol. 26(10):631-5.

- **Geppert J**.; Demmelmair H.; Hornstra G.; Koletzko B.(2008). Co-supplementation of healthy women with fish oil and evening primrose oil increases plasma docosahexaenoic acid, gamma-linolenic acid and dihomo-gamma-linolenic acid levels without reducing arachidonic acid concentrations. Br J Nutr. ;99(2):360-9.

- **Gillin, E**., FAO Statistical Yearbook 2005-2006, Table B.6. FAOSTAT database of World Agriculture **2006**.

- **Haggans, C. J**.; Travelli, E. J.; Thomas, i.; Martini, M. C. and Slavin, J. L. (2000) The Effect of Flaxseed and Wheat Bran Consumption on Urinary

References

Estrogen Metabolites in Premenopausal Women. Cancer Epidemiol. Biomarkers Prev, 9**,** 719- 725.

- **Hallfrisch, J**.; Facn; Behall, K. M.(2000). Mechanisms of the effects of grains on insulin and glucose responses. ACN**,** 19, (3), 320S-325S.

- **Hancock, RE**.; and Sahl, HG. (2006). Antimicrobial and host-defense peptides as new anti- infective therapeutic strategies. Nat.Biotechnol, Vol. 24, (12): 1551-1557.

- **Harlan, J. R** .(1981). The early history of wheat: earliest traces to the sack of Rome. In Wheat Science - today and tomorrow, Evans, L. T.; Peacock, W. J., Eds. the Press Syndicate of theUniversity of Cambridge: Cambridge; 1-20.

- **Henz BM** .; Jablonska S.; van de Kerkhof PC. (1999). Double-blind, multicentre analysis of the efficacy of borage oil in patients with atopic eczema. *Br* J Dermatol;140:685-688.

- **Hernot, D. C** .; Boileau, T. W.; Bauer, L. L .; Swanson, K. S .; and Fahey, G. C., Jr. (2008).In vitro digestion characteristics of unprocessed and processed whole grains andtheir components. J Agric Food Chem, *56*(22), 10721-10726.

- **Helzlsouer, K. J**.; Huang, HY.; Alberg, AJ.; Hoffman, S.; Burke, A.; Norkus, EP .; Morris, JS .; Comstock, GW (2000). "Association Between alpha-Tocopherol, gamma-Tocopherol, Selenium, and Subsequent Prostate Cancer". J. Natl. Cancer Inst 92(24): 2018–23.

- **Hidve´gi** .;M., E. Ra´so´ .; R. To¨mo¨sko¨zi-Farkas .; S. Paku, K. Lapis ,; and B. Szende. (1998). Effect of FWGE + Vitamin C on tumor growth and metastasis in experimental animals. Anticancer Res. 18:3553–3358.

- **Horrobin DF**. (1993). "Fatty acid metabolism in health and disease: the role of delta-6-desaturase". Am. J. Clin. Nut*r*. **57** (5): 732S–736S.

- **Hutter, JJ**. (2010). "Childhood leukemia.". Pediatrics in review / American Academy of Pediatrics 31 (6): 234–41.

References

- **Hsu, L.-C** .; Hsu, Y.-W .; Liang, Y.-H .;Liaw, C.-C., Kuo, Y.-H .; and Pan, T.-M. (2012). Induction of Apoptosis in Human Breast Adenocarcinoma Cells MCF-7 by Monapurpyridine A, a New Azaphilone Derivative from Monascus purpureus NTU 568. molecules, 664-673.

- **Iyer G** .; Hanrahan AJ .; Milowsky MI .; Al-Ahmadie H .; Scott SN.; Janakiraman M .; Pirun M .; Sander C .; Socci ND .; Ostrovnaya I .;Viale A.; Heguy A .; Peng L.; Chan TA .; Bochner B .;Bajorin DF .; Berger MF .; Taylor BS .; Solit DB. (2012) Genome sequencing identifies a basis for everolimus sensitivity. Science 338: 221.

- **Jakab F**.; Shoenfeld Y.; Balogh A.(2003). A medical nutriment has supportive value in the treatment of colorectal cancer. *Br J Cancer*; 89(3):465-469.

- **Jans, J**.; Garinis. GA.;Schul, W.; Van Oudenaren, A.; Moorhouse, M.; Smid, M.; Sert, YG.;Van Der Velde, A .;Rijksen, Y.; De Gruijl, FR.; Van Der Spek, PJ .; Yasui, A.; Hoeijmakers, JHJ .; Leenen, PJM .; Van Der Horst,GTJ (2006). "Differential Role of Basal Keratinocytes in UV-Induced Immunosuppression and Skin Cancer". Mol Cell Biol 26(22): 8515–8526.

- **James P. Tam** .; Yi-An Lu .; Jin-Long Yang .; and Kou-Wei Chiu.(1999). An unusual structural motif of antimicrobial peptides containing end-to-end macrocycle and cystine-knot disulfides," Proc. Natl. Acad. Sci. U.S.A, 96, (16):8913–8918.

- **Janet Raloff** (2012). "Tricks Foods Play". Science News Magazine 182 (7): 25–28.

Jensen SK.; *Lauridsen, C (2007). "*Alpha-tocopherol stereoisomers*"*.Vitam. Horm. 76*:* 281–308.

- **Jemal, A**.; Tiwari,Rc.;and Murray,.T.(2004),cancer statistics.CA Cancer j.Clin., 54,8-29.

- **Jones, J. M**.; Reicks, M.; Adams, J .; Fulcher, G .; and Marquart, L. (2004) Becoming proactive with the whole-grains message. (Food Science). NUTR. TODAY, 39, 10 -18.

References

- **Judson PL**,;Al Sawah E.; Marchion DC. (2012) . Characterizing the efficacy of fermented wheat germ extract against ovarian cancer and defining the genomic basis of its activity. Int J Gynecol Cancer.*Jul* ; 22(6):960-967.

- **Kahlon, T.S**.(**1989)**Nutritional implications and uses of wheat and oat kernel oil. Cereal Food Worls, *34*, 872-875.

- **Kawakami Y**.; Yumoto K.; Kawakami T.2007. An improved mouse model of atopic dermatitis and suppression of skin lesions by an inhibitor of tec family kinases. Allergol Int. 56:403–409.

- **King**, Michael W. (2013). "Introduction to the Eicosanoids". The Medical Biochemistry *Page*. 1996–2013 themedicalbiochemistrypage.org, LLC. Retrieved 23 July.

- **Kim, K**. (2012). "Neuroimmunological Mechanism of Pruritus in Atopic Dermatitis Focused on the Role of Serotonin**."** Biomol Ther 20(6): 506–512.

- **Koltay.A,** Daly. N. L,; Gustafson. K. R.; and Craik. D. J. (2005) "Structure of circulin B and implications for antimicrobial activity of the cyclotides," INT J PEPT RES THER, 11, (1): 99–106.

- **Krakowski AC**. (2008). Management of atopic dermatitis in the pediatric population. Pediatrics, 122(4): 812–824.

- **Krings, U**.; Berger, R.G.(2001**)**. Antioxidant activity of some roasted foods. Food Chem., *72*,223-229.

- **Krings, U**.; El-Saharty, Y.S.; El-Zeany, B.A.; Pabel, B.; Berger, R.G. 2000. Antioxidant activity ofExtracts from roasted wheat germ. *Food Chem., 71*, 91-95.

- **Kumar, P**.; Yadava, R.; Gollen, B.; Kumar, S.; Verma, R.; and Yadav, S. (2011). Nutritional Contents and Medicinal Properties of Wheat: A Review. LSMR,22, 10 .

- Kushi L.; Giovannucci E.2002. Dietary fat and cancer. Am J Med ; 113 (9): 63S-70S.

References

- **Leenhardt F**.; Fardet A .; Lyan B .;Gueux E.; Rock E.; Mazur A.; Chanliaud E .;Demigné C.; Rémésy C.J.2008. Wheat germ supplementation of a low vitamin E diet in rats affords effective antioxidant protection in tissues.Am Coll Nutr.; 27(2):222-8.

- **Liu YJ**.; Cheng CS.; Lai SM .;Hsu MP.; Chen CS.; Lyu PC. (2006). "Solution structure of the plant defensin VrD1 from mung bean and its possible role in insecticidal activity against bruchids," Proteins, 63 (4):777–786.

- **Marcsek Z** .; Kocsis Z.;Jakab M.; Szende B.; Tompa A.(2004). The efficacy of tamoxifen in estrogen receptor-positive breast cancer cells is enhanced by a medical nutriment. Cancer Biother Radiopharm. 19(6):746–753.

- **Matsuoka K**.; Shitara H.; Taya C.;Kohno K.; Kikkawa Y .;Yonekawa H. (2003) 'Novel basophil- or eosinophil-depleted mouse models for functional analyses of allergic inflammation.', PLoS One, 8(4):609-758

- **McKenna SP** .; Doward LC. (2008). Quality of life of children with atopic dermatitis and their families. Curr Opin Allergy Clin Immunol 8(3):228-31.

- **Meduri NB**.; Vandergriff T.; Rasmussen H.; Jacobe H. (2007). "Phototherapy in the management of atopic dermatitis: a systematic review". Photodermatol Photoimmunol Photomed 23 (4): 106–112.

- **Melnik BC**, Plewig G .(1989). Is the origin of atopy linked to deficient conversion of omega-6-fatty acids to prostaglandin E1? J Am Acad Dermatol; 21:557–63.

- **Miller, H. E**.; Rigelhof, F.; Marquart, L.; Prakash, A.; Kanter, M., Antioxidant content of whole grain breakfast cereals, Yu, L.; Haley, S.; Perret, J.; Harris, M.; Wilson, J.; Qian, M.(2002).Free radical scavenging properties of wheat extracts. J.agric.food chem**,** 50, (6), 1619-1624.

- **Miyake JA**. 2009. Gamma-linolenic acid inhibits both tumour cell cycle progression and angiogenesis in the orthotopic C6 glioma model through changes in VEGF, Flt1, ERK1/2, MMP2, cyclin D1, pRb, p53 and p27 protein expression. Lipids.Health Dis.; 17.8.

References

- **NCI.** Retrieved 13 June 2014.

- **Osborne, T. B**.; and Harris, I. F. (1906). The chemistry of the protein bodies of the wheatkernel--part II. Am. J. Physiol., *17*(3), 223-230.

- **Palmer, CN**.; Irvine, AD.; Terron-Kwiatkowski, A.; Zhao, Y.; Liao, H.; Lee, SP.; Goudie, DR.; Sandilands, A.; Campbell, LE.; Smith, FJ.; O'Regan, GM.; Watson, RM.; Cecil, JE.;Bale, SJ.; Compton, JG.; DiGiovanna, JJ.; Fleckman, P.; Lewis-Jones, S.; Arseculeratne, G.;Sergeant, A.;Munro, CS.; El Houate, B.; McElreavey, K.; Halkjaer, LB.; Bisgaard, H.; Mukhopadhyay, S.; McLean, WH (2006). "Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis.".Nature Genetics 38 (4): 441–6.

- **Padovan L**.; Scocchi M.; and Tossi A. (2010). "Structural aspects of plant antimicrobial peptides," Curr. Protein Pept. Sci., 11, (3):210–219.

- **Pelegrini PB**.; Murad AM.; Silva LP.; Dos Santos RC.; Costa FT.; Tagliari PD.; Bloch C Jr.; Noronha EF.; Miller RN.; Franco OL.; (2008). "Identification of a novel storage glycine-rich peptide from guava (Psidium guajava) seeds with activity against Gram-negative bacteria," Peptides, 29, (8), 1271–1279.

- **Pelegrini PB**.; Franco OL. (2005) "Plant γ-thionins: novel insights on the mechanism of action of a multi-functional class of defense proteins," International Journal of Biochemistry and Cell Biology, 37, (11), 2239–2253.

- **Piras, A**.; Rosa, A .; Falconieri, D.; Porcedda, S.; Dessì, M.; and Marongiu, B. (2009). Extraction of Oil from Wheat Germ by Supercritical CO2. molecules, 14, 2574-2581.

- **Prior, R. L**.; Wu, X.; Schaich, K .; Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. j.agric.food chem 2005**,** 53, (10), 4290-4302.

References

- **Ranum P** .; Wesley A. (2013).Fortification Handbook Vitamin and Mineral Fortification of Wheat Flour and Maizr Meal, 1.Micronutrient initiative,Ottawa.
- **Richmond**, C. (2003). "David Horrobin". BMJ 326 (7394): 885.
- **Rigotti A** (2007). "Absorption, transport, and tissue delivery of vitamin E". Mol. Aspects Med. 28 (5–6): 42336.
- **Saether O**.; Craik DJ.; Campbell ID.; Sletten K.; Juul J and.; Norman DG. (1995). "Elucidation of the primary and three-dimensional structure of the uterotonic polypeptide kalata B1," Biochemistry, 34, (13), 4147–4158.
- **Saiko P.;** Ozsvar-Kozma M.; Graser G.; (2009): Avemar, a nontoxic fermented wheat germ extract, attenuates the growth of sensitive and 5-FdUrd/Ara-C cross-resistant H9 human lymphoma cells through induction of apoptosis. *Oncol Rep.*; 21(3):787-791.
- **Saiko P**, Ozsvar-Kozma M, Madlener S, 2007. Avemar, a nontoxic fermented wheat germ extract, induces apoptosis and inhibits ribonucleotide reductase in human HL-60 promyelocytic leukemia cells. *Cancer Lett.* 8;250(2):323-328.
- **Salinas, R**.1993**.** Alimentos Grasos y Nutrición, Bromatología Aplicada a la Salud; El Ateneo: Buenos Aires, Argentina,; Chapter 7.
- **Sharma S**.; Stutzman JD.; Kelloff GJ.; Steele VE.1994. Screening of potential chemopreventive agents using biochemical markers of carcinogenesis. Cancer Res; 54:5848-55.
- **Shai Y**., "Mode of action of membrane active antimicrobial peptides," Biopolymers, 66, (4), 236–248, 2002.
- **Shewry, P**. (2009). Wheat. J Exo bot, *60*, 1537-53.
- **Shuler, P.**1990. Natural antioxidants exploited commercially. In: Food Antioxidants; Hudson, B.J.F, Ed.; Elsevier Applied Science: London, UK,; Chapter 4.

References

- **Sitaram N**.;Nagaraj R. (1999). "Interaction of antimicrobial Peptides with biological and model membranes: structural and charge requirements for activity," Biochimica et Biophysica Acta, . 1462, (2): 29–54.

- **Slavin, J. L**.; Jacobs, D.; Marquart, L. **2001**. Grain processing and nutrition. critical rev biotech**,** 21, (1), 49-66.

- **Slavin, J**. 2003**.**Why whole grains are protective: biological mechanisms. P NUTR SOC**,** 62, (1), 129-134.

- **Smith, R**. (2003). "The drugs don't work". BMJ 327 (7428):, W. (1996) Drug Prototypes and Their Exploitation. Wiley, UK.

- **Srivastava, L. M.** (2002). Plant growth and development*;* hormones and environment.San Diego: Academic Press.

- **Sukkar SG**.; Cella F.; Rovera GM .; Nichelatti M .; Ragni G .; Chiavenna G .; Giannoni A,Ronzani G .; Ferrari C. A.(2008). multicentric prospective open trial on the quality of life and oxidative stress in patients affected by advanced head and neck cancer treated with a new benzoquinone-rich product derived from fermented wheat germ (Avemar), Mediterr JNutr Metab, 1,(1):37-42

- **Sprangers MC**.;Lakhai W.; Koudstaal W.; Verhoeven M.; Koel BF.; Vogels R.; Goudsmit J.; Havenga MJ.; Kostense S. (2003) 'Quantifying adenovirus-neutralizing antibodies by luciferase transgene detection: addressing preexisting immunity to vaccine and gene therapy vectors', J Clin Microbiol, 41 (11), 5046-52.

- **Steward JCM**.; Morse PF.; Moss M .; Horrobin DF.; Burton JL.; Douglas WS.; Gould DJ.; Grattan CEH.; Hindson TC.; Anderson J.; Janse´n CT, Kennedy CTC .; Lindskov R.; Strong AMM.; Wright S. (1991).Treatment of severe and moderately severe atopic dermatitis with evening primrose oil (Epogam): amulticentre study. J Nutr Med 2,(1).9_15.

- **Sneader W**.; Drug Prototypes and Their Exploitation. Chichester: Wiley; 1996.

References

- **Taube S**.; Oh DY .; Hamouda O. (2008) A functional toll-like receptor 8 variant is associated with HIV disease restriction. J Infect Dis;198:701–709.

- **Telekes A**.; Hegedus M.; Chae CH. (2009). Avemar (wheat germ extract) in cancer prevention and treatment. *Nutr Cancer*. 61(6):891-899.

- **Telekes A**, Kiss-Toth E, Nagy T: (2005). Synergistic effect of Avemar on proinflammatory cytokine production and Ras-mediated cell activation. *Ann N Y Acad Sci*, 1051:515-528.

- Terras FR .; Eggermont K.; Kovaleva V.; Raikhel NV.; Osborn RW.; Kester A, Rees SB.; Torrekens S.; Van Leuven F.; Vanderleyden J.1995. "Small cysteine-rich antifungal proteins from radish: their role in host defense," Plant Cell, 7, (5):573–588.

- **Tejeda M** .; Gaal D .; Szucs I.(2007). Avemar inhibits the growth of mouse and human xenograft mammary carcinomas comparable to endocrine treatments. *ASCO Meeting Abstracts*. 21, 25(18):21132

- **Tintle S** .; Shemer A .; Suárez-Fariñas M .; Fujita H .; Gilleaudeau P.; Sullivan-Whalen M.; Johnson-Huang L.; Chiricozzi A.; Cardinale I .; Duan S.; Bowcock A .; Krueger JG.; Guttman-Yassky E.2011. Reversal of atopic dermatitis with narrow-band UVB phototherapy and biomarkers for therapeutic response. J Allergy Clin Immunol.;128(3):583-93.

- **Traber MG**.; Packer L.(1995). Vitamin E beyond antioxidant function. Am J Clin Nutr; 62:1501S-9S.

- **Tran K**, Chan A.(1990). RRR-alpha tocopherol potentiates prostacyclin release in human endothelial cells. Evidence for structural specifity of tocopherol molecule. Biochim Biophys Acta; 1043:189-97.

- **Truswell, A. S**. (2002). Cereal grains and coronary heart disease. Eur J Clin Nutr**,** 56, (1), 1-14.

- **Turnidge JD** .; Ferraro MJ.; Jorgensen JH. (2003). Susceptibility Test Methods: General Considerations. In PR Murray, EJ Baron, JH Jorgensen, MA Pfaller, RH Yolken. Man.Clini.Microbiol. 8th Ed. Washington. American Society of Clinical Microbiology. 1103 ISBN 1-55581-255-4.

References

- **Van Gool CJ** .; Thijs C .; Henquet CJ.(2003). Gamma-linolenic acid supplementation for prophylaxis of atopic dermatitis – a randomized controlled trial in infants at high familial risk. Am J Clin Nutr:77:943-951.

- Varothai, S .; Nitayavardhana, S .; Kulthanan, K (2013). "Moisturizers for patients with atopic dermatitis." . Asian Pac J Allergy Immunol 31 (2): 918.

- **Venn, B. J**.; Mann, J. I. (2007). Cereal grains, legumes and diabetes. Eur J Clin Nutr**,** 58, (11), 1443-61.

- **Williams, H. C** (2003). "Evening primrose oil for atopic dermatitis: Time to Say Goodnight". BMJ **327** (7428): 1358.

- **Williams, Hywel** (2009). Evidence-Based Dermatology. John Wiley & Sons. p. 128.

- **Wiseman M**:(2008). The second World Cancer Research Fund/American Institute for Cancer Research expert report. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. Proc Nutr Soc; 67 (3): 253-6.

- **Witkowska. D**.; Bartyś .A and Gamian. A. 2008 "Defensins and cathelicidins as natural peptide antibiotics,"Postępy Higieny i Medycyny Doświadczalnej. 62. 694–707.

- **Wood , R**.; segall, N.; Ahlstedt , S.; and williams , P. (2007). Accuracy of IgE antibody laboratory results. Ann Allergy Asthma Immunol, 34-41.

World Cancer Report 2014. World Health Organization. 2014. pp. Chapter 5.13.

- **Yarbrough, KB .**; Neuhaus, KJ.; Simpson, EL (2013). "The effects of treatment on itch in atopic dermatitis". Dermatologic Therapy 26 (2): 110–119..
- **Yeaman MR**, Yount NY:(2003). Mechanisms of antimicrobial peptide action and resistance," Pharmacoloy Reviews, 55: 27–55.

- **Zock PL.**2001. Dietary fats and cancer. Curr Opin Lipidol; 12 (1): 510.

(الملخص)

هدفت هذه الدراسه للتحري عن تأثير تثبيط مستخلصات جنين القمح المحلي على بعض الاحياء المجهرية ، فضلا عن تأثيرها على خطوط ألخلايا السرطانية، والتهاب الجلد التأتبي (الأكزيما),في الفئران المخبرية فقد تم دراسة تحقيق ألكشف ألكمي والنوعي لكلى مستخلص الكلوروفورم ومستخلص الكلوروفوم /ميثانول باستخدام مطياف الغاز اللوني الشامل (GC-MS) للكشف عن نتائج مجموعات نشطة، وأظهرت وجود حمض اللينوليك مع نسبة 40٪، وكذلك وجود كل من حمض الأوليك وحمض octadecanoice وحمض hexadecanoic ووجود عدد كبير من الاحماض الامينيه التي قد تلعب دورا رئيسيا هاما جدا في كل تقييم النشاط الحيوي في هذا المشروع.

وقد تم اختبار تأثير المركبات المستخلصة على الميكروبات من مستخلص جنين القمح الخام (الميثانول /والكلوروفورم والميثانول) على 12 سلالة من البكتيريا الموجبة الغرام و السالبة الغرام واثنين من سلالات الفطريات لإيجاد الحد الأدنى للتركيز المثبط المناسب (MIC) ,فقد اثبتت الدراسه ان هنلك فعاليه كبيره لمستخلص جنين القمح لكلا طريقتي الاستخلاص على نمو سلالات البكتيريا والفطر بمعدل (MIC)يتراوح بين .0.165, 2.5 ملغرام/مل.

ودرس تأثير النشاط ألسمي لمسخلصات جنين القمح على خط خلايا سرطان الدم (k562) وخط خلايا سرطان الثدي(MCF-7) ، وأيضا مقارنتها على خط الخلية العادي (خلايا ليفية)، وكشفت النتائج النشاط السمي الكبير للمستخلص جنين القمح على خطوط الخلايا خلال فترة 72 ساعه من التعرض لتركيزات (900، 450، 225 و 112.2 ميكروغرام / مل) بالمقارنة مع طبيعية خط الخلية الليفية (الخلايا اليفية) , التي لم يظهر اي نشاط سمي على للتراكيز العاليه من المستخلص .

هذا وتم تحضير مرهم من مستخلص جنين القمح تم إعداده واختباره كعلاج للفئران ألمصابة بألتهاب ألجلد التأتبي (الأكزيما) موضعيا على حد سواء واستخدام المستخلص بصيغة مرم بتركيز 20٪ وثبت بعدها أن الحيوانات المختبريه المصابه بالاتهابات الأكزما قد تم معالجتها تماما بعد تعرضها للعلاج من مستخلص جنين القمح ومرهم جنين القمح 20 % بعد فتره 48 ساعه حيث اعطى نسبه مضادات ال IgE في عينات الدم المأخوذه من الفئران المختبريه التي أجريت عليها الدراسه بعد العلاج والتي حددتها النتائج ب (0.62 و 0.21)IgE على التوالي مقارنة مع البيتاميتازون 0.05٪ (0.21) IgE بعد 48 ساعة .

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين /كلية العلوم
قسم التقانة الاحيائية

تأثير مستخلصات جنين ألقمح على بعض الظواهر ألحيوية : دراسة داخل وخارج الجسم ألحي

رسالة

مقدمة الى مجلس كلية العلوم – جامعة النهرين وهي جزء من متطلبات نيل

شهادة ماجستير علوم في التقانة الاحيائية

من قبل

المعتصم بالله توفيق يونس
بكالوريوس تقانة احيائية وهندسه وراثية/جامعة فيلاديلفيا /ألأردن (2009)

بإشرف

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رمضان 1435 **تموز 2014**