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BOLU ABANT İZZET BAYSAL UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
Department of Chemistry

EVALUATION OF THE BIOCHEMICAL PROPERTIES OF *MORUS ALBA* FRUIT EXTRACT AND ITS HEALING EFFECT ON PATIENTS WITH ECZEMA

MASTER OF SCIENCE

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BOLU, HAZİRAN - 2023

APPROVAL OF THE THESIS

EVALUATION OF THE BIOCHEMICAL PROPERTIES OF *MORUS ALBA* FRUIT EXTRACT AND ITS HEALING EFFECT ON PATIENTS WITH ECZEMA submitted by **Maryam Sabeeh MADHLOOM** and defended before the Examining Committe Members listed below in partial fulfillment of the requirements for the degree of **Master of Science** in **Department of Chemistry, Institute of Graduate Studies of Bolu Abant Izzet Baysal University in 6.06.2023** by

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Maryam Sabeeh MADHLOOM

ABSTRACT

EVALUATION OF THE BIOCHEMICAL PROPERTIES OF *MORUS ALBA* FRUIT EXTRACT AND ITS HEALING EFFECT ON PATIENTS WITH ECZEMA
MSC THESIS
MARYAM SABEEH MADHLOOM
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INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF CHEMISTRY
(SUPERVISOR: ASSOC. PROF. DR. ERCAN SELÇUK ÜNLÜ)
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BOLU, JUNE 2023
xiii + 59

Eczema is considered one of the chronic diseases spread globally. In this study, the ointment was prepared from the white mulberry plant (*Morus alba*), which contains vitamin E. 200g of dried mulberry, grind and extracted with 96% ethanol, 60^{o}C in reflux setup, filtered in whatmann filter paper and evaporated with rotovap, using separation funnel suppled with butanol, after acquiring two phases, butanol phase was evaporated to obtain dried extract. Biochemical content of the extract was evaluated via gas chromatography analysis. Water-in-oil (W/O) emulsification was used to prepare ointment that contains 0.5g extract for each individual. The number of individuals on whom the ointment was tested were 110 divided into 3 groups (group one with eczema, group two healthy and group three control) with different ages and genders to compare the results for a period ranging from 4 to 6 weeks. For each group, blood sample were collected to be analyzed (Vitamin E, Granzyme B and Lipid profile). The antibacterial activity of the extract was tested against *S. aureus* at different concentrations which was detected on the skin surface of eczema patients. The results indicate that 0.5% of the extract was effective on eczema patients with slight changes in the blood analysis results, vitamin E were enhance from 3.776 µg/ml to 5.681 µg/ml, granzyme B were significantly lowered from 180.06 pg/ml to 141.09pg/ml and lipid profile slightly changed in patients. The antibacterial activity of the extract against *S. aureus* was recorded and different inhibition zones were observed (9mm to 16mm). This study shows that mulberry abstract can be used effectively as a supplement for eczema treatment.

KEYWORDS: Extraction, *Morus alba*, *Staphylococcus aureus*, Granzyme B, Vitamin E, Ointment, Eczema, Mulberry, Lipid profile

ÖZET

MORUS ALBA MEYVE EKSTRESİNİN BİYOKİMYASAL ÖZELLİKLERİNİN VE EGZAMALI HASTALARDA İYİLEŞTİRİCİ ETKİSİNİN DEĞERLENDİRİLMESİ
YÜKSEK LİSANS TEZİ
MARYAM SABEEH MADHLOOM
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BOLU, HAZİRAN - 2023
xiii +59

Egzama, küresel olarak yayılan kronik hastalıklardan biri olarak kabul edilir. Bu çalışmada E vitamini içeren beyaz dut bitkisinden (*Morus alba*) merhem hazırlanmıştır. Bu amaçla 200 gr kurutulmuş bitki öğütülerek %96 etanol ile 60^{o}C refluksta ekstrakte edilmiş, filtre edilmiş ve ayırma hunisi kullanılarak rotovap ile buharlaştırılmıştır. Bütanol kullanılarak iki faz elde edildikten sonra bütanol fazı buharlaştırılarak kurutulmuş ekstrakt elde edilmiştir. Ekstraktın biyokimyasal içeriği, gaz kromatografi analizi ile değerlendirilmiştir. Her hasta için %0.5 ekstrakt içeren merhem hazırlamak için yağ içinde su (W/O) emülsifikasyonu kullılmıştır. Merhemin denendiği 110 hasta, farklı yaş ve cinsiyette 3 gruba (birinci grup egzamalı, ikinci grup sağlıklı ve üçüncü grup kontrol) ayrılarak, 4 ila 6 haftalık bir süre boyunca sonuçları karşılaştırılmıştır. Her gruptan E Vitamini, Granzim B ve Lipit profili analizi için kan örneği alınmıştır. Ekstraktın antibakteriyel aktivitesi, egzama hastalarının cilt yüzeyinde farklı konsantrasyonlarda bulunan *S. aureus*'a karşı test edilmiştir. Sonuçlar incelendiğinde, %0,5'lik ektrakt kan analizi sonuçlarında hafif değişikliklerle egzama hastalarında etkili olduğu, E vitamini seviyelerinde (4'ten 5'e) farklı olduğunu, granzim B'nin (200'den 180'e) önemli ölçüde düşürüldüğünü ve lipid profilinin hafifçe değiştiğini görülmüştür. Ekstraktın *S. aureus*'a karşı antibakteriyel aktivitesi olduğu da gözlenmiş olup ve farklı inhibisyon bölgeleri (9 mm ila 16 mm) tespit edilmiştir. Bu çalışma dut özünün egzama tedavisinde destekleyici olarak etkili bir şekilde kullanılabileceğini göstermektedir.

ANAHTAR KELİMELER: Ekstraksiyon, *Mors alba*, *Staphylococcus aureus*, Granzim B, E vitamini, Merhem, Egzama, Beyaz dut, Lipid profili

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LIST OF ABBREVIATIONS AND SYMBOLS

AD : Atopic Dermatitis

GC MASS : Gas Chromatography Mass Spectrometry

Gr. B : Granzyme B

FA : Fatty Acid

HDL : High –density- lipoprotein

K : Potassium

LDL : Low density lipoprotein-cholesterol

MBC : Minimum Bactericidal Concentration

MIC : Minimum Inhibitory Concentration

MRSA : Methicillin-resistant Staphylococcus aureus

N : Nitrogen

NACl : Sodium Chloride

P : Phosphorus

PGE2 : Prostaglandin E2

ROC : Receiver Operating Characteristic

ROTOVAP : Rotary Evaporator

S. aureus : Staphylococcus aureus

TC : Total cholesterol

TG : Triglyceride

ACKNOWLEDGEMENTS

I would like to thank my esteemed supervisor Assoc. Prof. Dr. Ercan Selçuk ÜNLÜ and co-supervisor Assist. Prof. Dr. Ekhlass M. TAHA for their guidance, advice, criticism and encouragement throughout the research.

I like to do a special thank for my supervisor Assoc. Prof. Dr. Ercan Selçuk ÜNLÜ for his notes, comments, and his patience with me.

I would also like to thank co-supervisor Assist. Prof. Dr. Ekhlass M. TAHA for her support during the clinical work in the lab.

I would like to thank Dr. Ömer Can ÜNÜVAR for his help during the extractions.

I must express my sincere gratitude to my family for their support during the study period.

I would like to extend my special thanks to my sister Assist. Lecturer Ghada SABEEH for her encouragement and support.

1. INTRODUCTION

1.1 Atopic Dermatitis

Atopic dermatitis (AD), also known as eczema, is a very common non-infectious skin disease, and it affects many people worldwide. Even though it is not contagious along with diabetes, cystic fibrosis, and epilepsy, it is one of the world's most prevalent chronic diseases[1,2,3,4-5]. AD is caused by genetic and environmental factors that results with changes on the skin and immune system[1].

This chronic disease begins with dry skin, eczematous lesions, and lichen formation[2]. AD has significant implications for diagnosis and treatment methods due to appearance of multiple illnesses, this includes allergic symptoms, respiratory disease, skin disease, food allergy, neuropsychiatric disorders, other inflammatory and autoimmune diseases, lymphomas, and cardiovascular diseases[1,2,5,6,7-8]. Age, chronic disease and the severity of illness are the factors affecting the type of Eczema treatment[2].

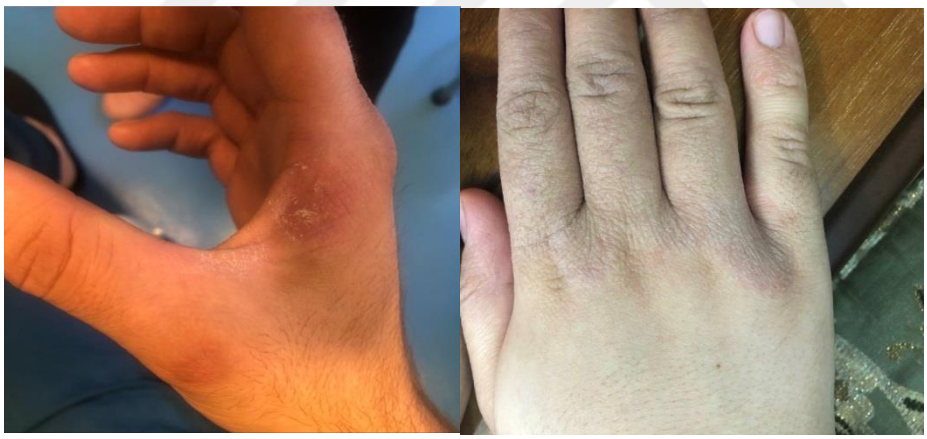

Figure 1.1 Patient with Eczema (Taken by Maryam Sabeeh)

1.1.1 Etiology

Atopic dermatitis is caused by environmental and genetic factors that influence the skin and immune system[9,10]. AD is a part of atopic triad. Atopic triad includes eczema, allergic rhino conjunctivitis and asthma. These are sometimes present in the same individual and occur simultaneously or one after the other. Atopic children are more likely to exhibit atopic symptoms as compared to non-atopic children. For children whose parents are atopic, there is a greater than 50% chance that they will be atopic. Additionally, up to 80% of offspring can potentially

be atopic. In the case of both parents being affected by atopy, there is a greater than 80% chance that their child will exhibit atopic symptoms[9,10]. Diseases have specific causes, which a doctor can discover by examining patients' medical history and examining their current symptoms. This can help explain why some people develop certain diseases more often than others. Factors that can affect disease severity include exposure to bacteria, weather, pollution, personal care products, food, or other external factors[9].

Focus on developing yourself to help avoid or remedy the issue. Patients require guidance on the best treatment options and a health diagnosis, they also need advice on avoiding irritants in their life[10]. Having a family history of AD disease greatly increases one's risk of developing the disease[2,9,13]. Other major risk factors associated with AD are living in an urban area and being exposed to air pollutants and low ultraviolet light. Additional risk factors include having a diet high in sugar and poly unsaturated fatty acids and regularly being exposed to antibiotics. Main symptoms typically appear before age five[2,9,14]. Long-term effects of stress, antibiotics, alcohol and tobacco on a mother's or postpartum state, includes the effects of both short-term and long-term exposure. Giving regular vaccinations to children enhances their immunity to viral or bacterial infections and breastfeeding also strengthens a child's immunity. Airborne pollution, the working environment and having a pet developing the risk of AD[2,13,10].

S. aureus colonizes approximately 90% of Atopic dermatitis patients, besides the roles of other organisms, including yeast, for example *Malassezia* spp., which can directly stimulate skin inflammation[2,15].

1.1.2 Epidemiology

Atopic dermatitis is estimated to affect at least 230 million people, according to the World Health Organization's Global Burden of Disease Initiative by being the leading cause of non-fatal disease burden in skin diseases[2,16]. AD affects many people around the world, and it is a common chronic disease of the skin that affects developed countries and all age groups both male and female, young and old, it is also unisex[2,17,18,19]. It is estimated that 15% to 25% of children younger than age 6 have the condition, referred to as atopic childhood disorder because it is often found in families with bronchial asthma and/or allergic rhinitis. AD in adults occurs at 1% to 10% frequency [2,20,17,18,21-13].

AD is a long-term chronic disease, for a while at the site of the injury we can notice relapses, calmness and lack of irritation also can appear[2]. Early research had suggested that the disease clears in more than > 50% of affected children, while severe cases persist into childhood[9].

Opinions regarding the cross-section of the public are gathered via web-based regionally performed polls. These were performed in the US, Canada, France, Germany, Italy, Spain, the UK and Japan. The point prevalence of adult AD in the overall population is 4.9% in the US, 4.4% in the European Union, 3.5% in Canada and 2.1% in Japan[22].

The disease is widespread in the world, but it can be controlled, and its symptoms and treatment last for many long years.

1.1.3 Skin Barriers

Undermining of the cutaneous barrier is a main pathogenic agent of atopic dermatitis. The role of the cutaneous barrier in the pathogenesis of atopic dermatitis disease has been a matter of discussion for a while[23,24]. In this context, (pro-)filaggrin is the significant partition protein encoded by in the gene filaggrin (FLG) that has the strongest genetic combination with atopic dermatitis so far for loss-of-function mutations[25].

The FLG null mutations R501X and 2282del4 were repeatedly linked with the European Caucasian AD patients. Moreover, AD patients that are suffering from acute forms of AD with chronic path because of loss-of-function mutations in the FLG gene[26,27]. In the FLG mutation carriers the risk of eczema raises exposure to specific environmental allergens. AD and asthma develop because of the FLG mutations[28].

FLG plays various roles in pathophysiology of atopic dermatitis which clarify why decrease expression of an individual component of the epidermal differential complex might possess large effect on the complete function of the skin barrier.

FLG collect keratin filaments into tight bunches and modifies the synthesis of keratinocytes and the granular cell coat, so it reacts with lamellar bodies and decreased availability of filaggrin metabolites, such as natural moisturizing factor, that conduct to changes of skin hydration and skin pH[29,30].

Lower ceramide attenuates the activity of enzymes responsible for ceramide composition by increasing skin pH that promotes activity of serine proteases and

kallikreins. This leads to dissolution of corneo desmosomes and intercellular adhesion. Eventually, these mechanisms result in raised Th2 inflammation and increased permeation of allergens during the skin[31,32].

Genetics as well as practical studies on FLG mutations have demonstrated that a genetically predetermined skin barrier dysfunction appears as a highly dangerous agent for atopic dermatitis[33,34].

Immunologic factors effect on skin barrier action in AD patient as well as the other genetic factor also observable independently from the FLG genotype which are the factors that effect on modulate filaggrin expression secondarily[33,34].

In addition to loss of function mutations in FLG gene, another factors, such as environmental factors including skin irritation and mechanical damage, DNA methylation state or variations of FLG copy numbers, low humidity, the cytokine milieu in the skin with reduction of filaggrin expression by Th2 cytokines, IL-17, IL-22, IL-25 or IL-31 and also the microorganisms colonizing the skin and topical and systemic treatment[30].

Skin barrier dysfunction in AD patients caused by raises in Th2 cytokines increase serine protease kallikrein 7 (KLK7) levels[35].

Not just the skin oneself, but microorganisms that already settled the skin represent the initial line of the skin barrier[36,37].

The pathogenesis of AD occurs when the skin immune system interacts with microbes[36,37].

Due to various agents, increase in pH and a Th2-dominated micro milieu high quantities of *Staphylococcus aureus* were discovered on atopic dermatitis skin[36,37].

S. aureus inhibits skin barrier action by releasing virulence agents such α-toxin to induce cell kill of keratinocytes. Genetic and immunologic as well as mechanical factors such as scratching induce skin barrier injury, allowing contact of skin resident antigen-presenting cells to allergens, bacterial and viral antigens in addition to other environmental factors. Activated antigen-presenting cells emigrate to lymph nodes and prime naive T cells to Th2 cells. High Th2 cytokines together with TNF-a and IFN-c further injure skin barrier functions by inducing apoptosis of keratinocytes as well as impair the function of tight junctions and promote Th2 responses by enhancing TSLP expression of epithelial cells. Furthermore, colonizing pathogens such as Staphylococcus aureus weaken barrier function

through the release of virulence factors to induce keratinocyte death and to boostTh2-type inflammation. Together with genetical and immunological factors contribute to skin barrier dysfunction and play a major role in the pathogenesis of Atopic dermatitis[38] (Figure 1.2).

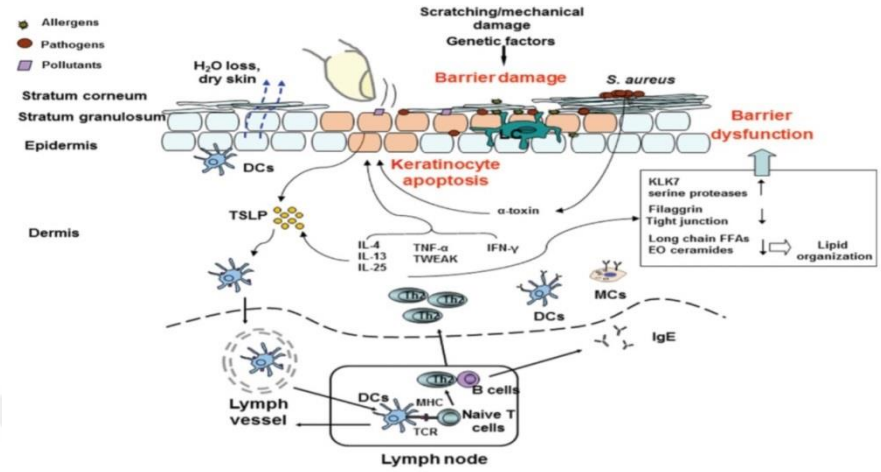

Figure 1.2 Graphic synopsis of effects of skin barrier on the pathogenesis of AD.

1.1.4 Pathophysiology

Patients with atopic dermatitis have defects in skin barriers. Xerosis and environmental irritants and allergens lead to inflamed, itch and classic clinical result for AD patients[39,40]. Ceramide is a sphingolipid in cornea stratum responsible to the skin barrier function and prevention percutaneously dehydration[39.40].

The deficiency of skin barrier cause irritants and allergens to penetrate the skin and is the reason of inflammation via an overactive Th2 response (elevation of IL-4, IL-5 cytokines) Lesion and Th1 response (including IFN-γ and IL-12) Chronic Lesion[39.40].

Revocation or scratching of the skin releases keratinocytes also associated with inflammation are cytokines such as TNF-α, IL-1 and IL-6.

Lowering the antibacterial peptides and a reducing agent, (Human β-defensins, cathelicidins), are found inside skin cells of AD[39.40].

1.1.5 Prognosis

In general, there are many patients getting better AD over time. Nevertheless, AD patients with asthma and allergic rhinitis cannot be treated primarily when growing with AD from childhood, and it lasts for decades, and relapse often requires drug uses[40].

Pollen, pet hair, smoke, soap, detergent, and wool, as well as tobacco and smoking for whom exposed continuously will be suffering from all symptoms all times and life will not only be pricey, but also worrisome[39].

The symptoms commonly experienced by people with eczema make them more likely to avoid leaving home or going to work. Eczema is estimated to cost more than $5.5 billion annually in North America due to its impact on people's health and well-being," said David Granville, researcher, Institute for Health, press release.

Kaposi varicella form rash is multiples form of AD that is connected to epidemics like herpes. In AD area vesicular lesions spread and suddenly it distributed to all over the unaffected skin[20].

Patients with atopic dermatitis also became vulnerable to skin disease, *Staphylococcus* and *streptococcus sp.* are present[21].

1.2 *Morus alba* (white mulberry)

Morus alba is a plant (Figure 1.3), and it is commonly referred to as mulberry. It belongs to *Moraceae* family, the most popular three species are: M. *alba*, M. *rubra*, and M. *nigra*. Species can be binary or unary and small or medium size, in places like South Europe, North Africa, Arabia, China, and Japan[41].

According to tradition, history, and science, it is widely known that plants can be used to produce drugs. Especially because of their accessibility, plants-based drugs become a significant component of the healthcare systems. It has been found that *Morus* sp. are a source of phenol and flavonoids, because of their antioxidant properties. Among those, anthocyanins have significant biological, pharmacological, and structural significance[41].

Figure 1.3 White mulberry plant (Taken by Maryam Sabeeh)

1.2.1 Chemical Composition

There are 24 species and at least 100 known subspecies under *Morus* genus that are adapted to a wide variety of climates, topography, and soil conditions. Environmental conditions also influence the composition, which is characterized by the presence of compounds that are considered dietary supplements because they provide health benefits and play a role in the treatment of disease.[42] The most common species are *Morus alba* (white mulberry), *Morus nigra* (black mulberry) and *Morus rubra* (red mulberry) [42,43].

Morus alba has the highest pH and soluble solids, and has a sweeter taste, making it the most recommended for processing[42]. Regarding the physicochemical composition, the average weight of fruits of *M. alba* species range between 2.0 to 4.0 g, and the water content is high (about 70%). The lipid content of *M. alba* is slightly lower (1.1%), but the lipid content of *M. alba* is the highest among the three[42].

Fatty acid (FA) profiles of all species include linoleic (C18:2), palmitic acid (C16:0) and the oleic acid (C18:1) for *M. nigra* and *M. alba*[42,44].

In terms of protein content, *M. alba* has higher values (10.15-13.33%), suggesting that the mulberry plant is an excellent source of plant-based protein and contributes to the recommended daily protein intake[45].

Calcium, magnesium, iron, sodium, zinc, manganese, copper and selenium are extremely abundant in the fruit's minerals but in low concentrations. Additionally, N, K and P minerals are present in high quantities[42]. *M. alba* contains high levels of iron (119.3 and 241.8 milligrams per kilogram) as well as low levels of sodium. This plant is ideal for those on a low sodium diet (0.01 mg/100 g) [46].

Table 1.1 Description of the nutrients of the fruit of *Morus alba* [42].

Chemical Composition	Morus alba
Lipid (%)	1.10
Linoleic acid (%)	57.26
Palmitic acid (%)	22.42
Oleic acid (%)	10.49
Protein (%)	10.15-13.33
P (mg/100 g)	247
K (mg/100 g)	1668
Ca (mg/100 g)	152
Mg (mg/100 g)	106
Fe (mg/100 g)	4.2
Na (mg/100 g)	60
Mn (mg/100 g)	3.8
Zn (mg/100 g)	2.8
Cu (mg/100 g)	0.5
Se (mg/1000 g)	0.005
Vitamin C (mg/100 mL)	22.4

1.2.2 Phytochemicals

Morus alba locate mostly in subtropical regions of Asia, America, Europe, Africa, and India. This species, extensive geographic distribution range results in a significant degree of environmental variability that affects both its physical and chemical characteristics, particularly the profile of bioactive substances including anthocyanins, carotenoids, and flavonoids[42,47]. It is a deciduous plant with a height

range of 10 to 20 meters that is utilized in the design of gardens and urban landscapes, even to fortify sandstone[48]. Because of their high nutritional content (mostly protein) and tasty flavor, the leaves are produced in most Asian countries and are fed to both herbivores and silkworms[46].

Phytochemicals are significant antioxidants that have positive impacts on human health, particularly in the reduction of cancer, inflammation, and cardiovascular disease. Most of the phytochemical components are flavonoids in *M. alba*. A class of flavonoids called flavonols is present in the *M. alba* species. They are categorized into 20 species, with quercetin, kaempferol, and isorhamnetin being the three primary glycated forms that support the physiological functions of the plant[47].

The content of phenolic compounds and flavonoids varies according to the genetic characteristics of the species, environmental conditions, and maturity stage[42]. White mulberry is low in flavonoids and anthocyanins among other mulberry species.

Table 1.2 Description of the phytochemicals of the species *Morus alba*[42].

Phytochemicals	*Morus alba*
Total phenolics (mg GAE/100 g)	181
Total flavonoids (mg GAE/100 g)	29
Kaempferol (mg/g)	ND
Quercetin (mg/g)	0.0036

The leaves also have significant nutritional characteristics. The primary organic acids included in mulberry seeds are citric and malic acids, which had the greatest initial quantities (32.2-105.5 mg/100 g) respectively. Mulberry leaves may also be added to flour to boost the food's nutritional value and enhance its stability during storage due to their high protein content (13.4–19.4%)[46]. In vitro tests reveal antioxidant activity in *Morus* phenolic extracts. This was favorably linked with a total phenolic content of 5.55 mg GAE/g dry weight (DW) and a total flavonoid content of 16.96 mg rutin equivalent (RE)/g DW. Rutin, catechins, and chlorogenic acid are the three primary substances known to contribute to antioxidant capacity[49].

1.2.3 Toxicity of the *Morus alba*

Low cost and widespread availability made the researchers notes the efficacy of medicinal plants to cure sickness. However, their actions ensure that the toxicity danger is entirely eradicated. Hepatotoxicity is among the most typical issues. As a result, it is crucial to adjust the doses and monitor side effects in trials to provide the most benefits for individuals who take them without danger[50].

Tests on laboratory mice have shown that mulberry extract is not overtly toxic[51]. It is possible that the extract and dosage may not demonstrate acute toxicity given that continuous treatment for a week did not result in behavioral abnormalities such breathing alterations, weight loss, or death[52]. Higher dosages have also been reported by another research. Male and female rats were administered five different dosages of mulberry leaf ethanol extract four times over the course of 14 days (days 0, 10, and 14), with no fatalities at any dose (125, 250, 500, 1000, and 2000 mg/kg) [53].

The toxicity of ethanol extracts from *M. alba* at two doses (300 and 2000 mg/kg) was evaluated for 14 days in Swiss rats, the writers did not discover any animal fatalities or alterations in animal behavior, however, hematological analysis showed alterations. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), lymphocyte to monocyte ratio, and differential leukocyte fraction were all lowered by both extracts. They also demonstrated immune-system impacts[50]. In different research, the highest oral dosage of 2000 mg/kg resulted in liver alterations, reduced MCV and MCHC, and hematological abnormalities. On the other hand, studies have shown that the dosage of 300 mg/kg is not only safer but also more effective[54].

Genotoxicity studies showed no difference in micronucleated hyperstained erythrocyte counts at oral doses below 300 mg/kg compared to controls. In addition, studies showed that oral administration of mulberry extracted by ethanol is safe and an increase in micronuclei was observed[50]**.**

1.3 Granzym B

Granzyme B (Gr. B) is a multifunctional serine protease produce in cytotoxic T lymphocytes, and perforin is extracted from natural killer cells along with granzyme B for the induction of apoptosis[55].

Granzyme B has substantial role in the extracellular non-perforin-dependent environment of many inflammatory diseases, including some lung diseases,

cardiovascular diseases and skin diseases, promoting inflammatory responses, influencing extracellular matrix remodeling and inducing epithelial-mesenchymal transition and fibrosis[56].

New studies have confirmed that Gr. B is produced at high levels in skin lesions and peripheral blood of AD patients, and it is participatory in the disruption of AD barrier function, the evolution of inflammatory responses, increasing vascular permeability, and various other potential pathogenic mechanisms[57]. Yawalkar et al. showed significantly elevated expression of Gr. B in skin lesions than in healthy skin in eight patients with reasonable to acute AD[58]. Kamata et al. also showed elevated Gr. B concentrations in AD patients, it was positively correlated with indicators of AD severity[59]. Staining of normal skin, lesional atopic dermatitis is shown in Figure 1.4.

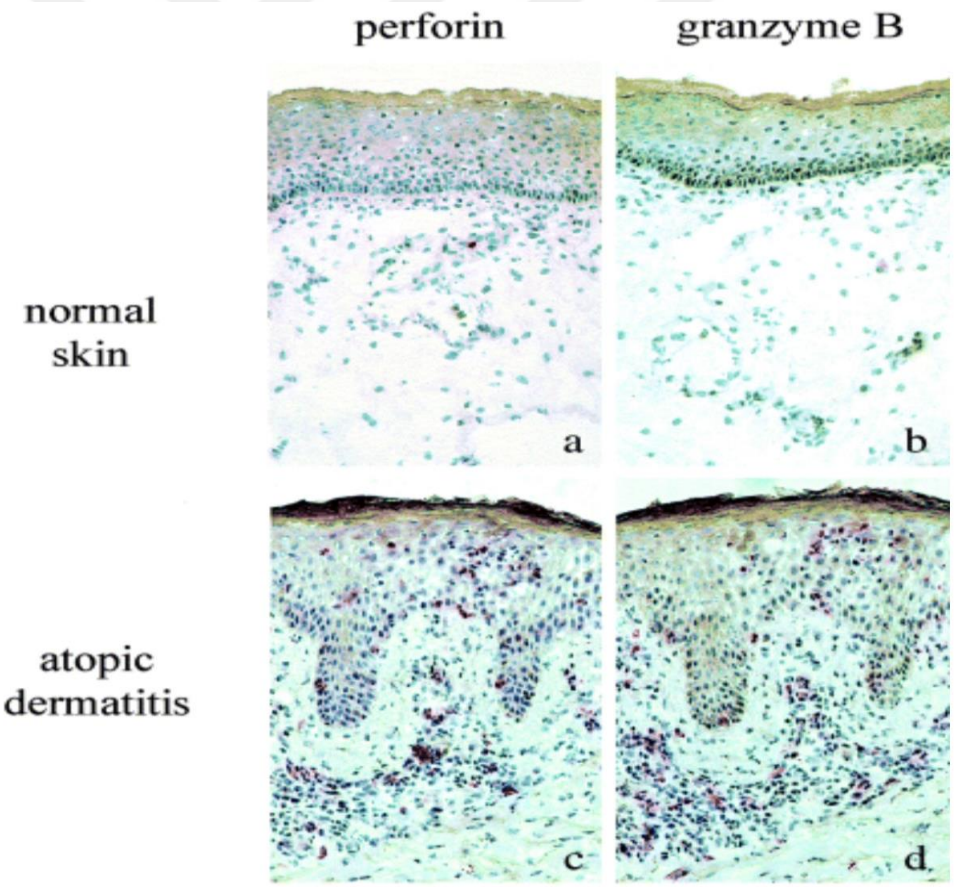

Figure 1.4 Perforin and granzyme B immunoreactivity is strongly enhanced in atopic dermatitis. Perforin and granzyme B immunoreactivity were barely detectable in normal skin (a,b). A marked enhancement of perforin and granzyme B immunostaining was observed in the mononuclear cell infiltrate in atopic dermatitis (c,d)[112].

These tentative results propose that Gr. B may play a crucial role during AD. The onset and evolution of AD are multifactorial, because of the three factors:

-Weakness skin barrier function

-T helper-mediated cellular immune balance

-Decrease skin microbial variety characterized by an raises proportion of *Staphylococcus aureus* colonization[60,61].

It is important to explain that these mechanisms do not cause the illness separately but react together, cause recurrent episodes and a chronic course of AD. In researches, Gr.B is primarily participate in pathogenesis of the first two aspects of AD.

In patients with atopic dermatitis, the skin's protective barrier is disrupted, making the skin more susceptible to foreign substances, this could lead to itchy, inflammation, dehydration and degeneracy in skin barrier[62].

Researchers found that eczema patients had stronger symptoms of itching when a key enzyme called granzyme B was present. Granzyme B breaks down skin proteins, making it easier for allergens to get through the barrier[63].

According to Granville, "There are proteins between the cells of our skin that hold them tight". He also reported that eating these proteins weakens these bonds, causing skin irritation and itching"[63].

Granzyme B levels in atopic dermatitis patients are correlated with pruritus and disease severity. With severe itching and rashes, eczema is now frequently treated with corticosteroid creams. However, these lotions can thin the skin over time, which can cause further damage and infection[62].

1.4 Vitamin E

Vitamin E (Figure 1. 5) is important fat-soluble antioxidant that has been used in dermatology for over 50 years. It is important ingredient in many cosmetics. Vitamin E fights free radical damage to the skin by protecting against various harmful effects of solar radiation. Several experiments have shown vitamin E to have anti-tumor and photo protective properties. There is evidence that vitamin E has some uses medically related to dermatology in the form of studies and clinical trials[64].

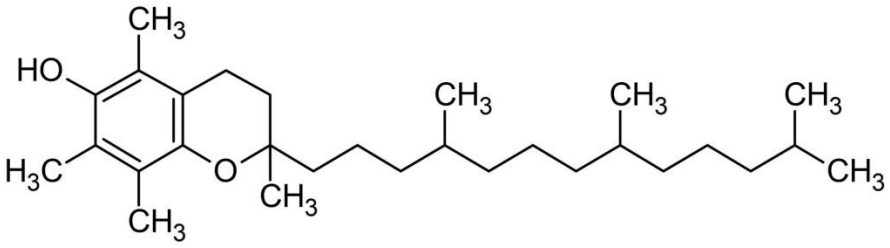

Figure 1.5 Vitamin E chemical structure [113].

Vitamin E formed about 0.32 mg\g from the dried powder of mulberry fruits, and a GC mass device used to examination of the dried fruit[64].

1.4.1 Historical perspective

Vitamin E was first introduced in 1922 by Katherine Bishop and Herbert M. Evans. Vitamin E biochemistry was explained in 1936 while its name was decided in Greek as tocopherol, meaning offspring and fertility[64,65].

1.4.2 Sources and forms of vitamin E

Vitamin E is derived from plants and can be found in vegetables, nuts, seeds, whole grains and oils. Some of the best sources are sunflower oil, olive oil, spinach and nuts[66].

Vitamin E contains eight distinct forms, each represented by a letter of the Greek alphabet. The most commonly occurring tocotrienol is γ-tocopherol, while α-Toc is the most common vitamin E derivative found in human tissues and serum. Additionally, β-Toc, γ-Toc and σ-Toc are other forms of vitamin E that are commonly found in human systems[66].

1.4.3 Vitamin E and epidermis: Molecular aspects

Levels of vitamin E lower the formation of sunburn cells, reduce edema caused by UVB rays, inhibit the production of PGE2 and nitric oxide, and protect the skin from oxidative stress[67,68]. This is because vitamin E lowers epidermal protection action levels from being set by oxidative stress in the body. Vitamin E also suppresses immune systems[69].

1.4.4 Stability of vitamin E

Vitamin E exists in many forms, but dl-α-Toc acetate is the most stable. Vitamin E naturally occurs in foods as α-Toc. It slowly oxidizes in the air, which allows it to be more stable when added to a topical preparation. Enzymes that combine vitamin E with tocopheryl esters make Vitamin E conjugates more stable and effective antioxidants[70].

Many cosmeceuticals use vitamin E and C in their formulations. When these topical treatments are not kept stable, little efficacy is achieved[70].

Vitamins E and C can reduce long-term UV damage as well as short-term UV aging when added to a high-stabilized formulation. They can also reduce UV skin cancer when added to a formulation that contains optimal antioxidants[71].

1.4.5 Dermatologic Indications

A study by Tsoureli-Nikita *et.al* reports a significant reduction in the severity of atopic dermatitis among patients treated with vitamin E. The single-blind, placebo-controlled study involved 96 patients diagnosed with atopic dermatitis, all were treated with vitamin E — an organic acid naturally found in nuts, seeds, vegetable oils and other plant foods — or placebo for 8 months. By the end of this time, patients' serum IgE levels had been improvement 62% in the vitamin E group compared to the placebo group. Vitamin E may be useful as a treatment option for atopic dermatitis as data suggests that high vitamin E intake, high IgE levels and symptoms of atopy are closely linked[72].

There are many benefits associated with topical vitamin E treatments. This includes the ability to reduce the appearance of skin conditions like acne and milia by inhibiting the formation of reactive oxygen species in the skin. Recommended levels for face creams contain between 0.5% and 1% vit. E[73].

Vit. E is an important vitamin for healing wounds and burns. There are very few clinical studies regarding Vitamin E's effectiveness in treating scars and burns[74,75].

1.5 Staphylococcus aureus

Staphylococcus aureus is Gram-positive bacteria stain purple by Gram stain that are cocci-form and tend to be organized as clusters that are characterized as “grape-like”. Colonies of S. aureus are represented in Figure 1.6. These organisms can grow in up to 10% salt on media, aureus means golden or yellow. Optionally, these organisms can grow aerobically or anaerobically and in 18 C to 40 C temperature[76,77].

Humans are the main reservoir for these organisms such as *S. aureus* (including drug-resistant strains such as MRSA) especially on the skin and mucous membranes[78,79] .

Estimation found that more than half of all adults are colonized, and about 15% of the people persistently carry *Staphylococcus aureus* in the anterior nares[77,80].

Some populations have raises in S. *aureus* colonization (up to 80%), such as health care workers, persons who use needles on a regular basis (i.e., diabetics), hospitalized patients, and immunocompromised individuals. *S. aureus* can be devolved from one to another by direct contact or by fomites[77,80].

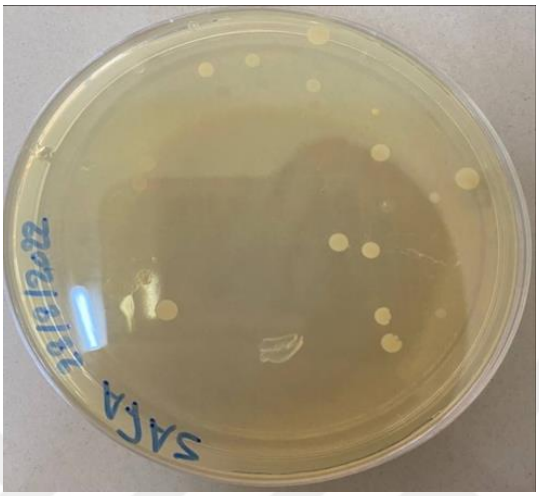

Figure 1.6 *Staphylococcus aureus* (Taken by Maryam Sabeeh)

1.5.1 The role of *S. aureus* in AD skin

A recent meta-analysis of 95 papers revealed that *Staphylococcus aureus* were discovered in about 70% of atopic dermatitis patients with skin lesions and 39% of AD patients without skin lesions[81]. *Staphylococcus aureus* is often reported in AD patients. *S. aureus* on the other hand, was rarely seen in skin that was healthy[82]. Similar to this, next-generation sequencing investigation of the skin microbiome showed that *S. aureus* was found in AD patients and that its frequency sharply increased during skin breakouts. Epidemiological monitoring has revealed that AD patients are more likely to have infectious impetigo, which is often brought on by *S. aureus* [83].

Staphylococcus aureus presence on biological surfaces is also known to vary in distribution. The variation of *S. aureus* colonization on AD skin may be connected to the particular dermatitis distribution seen in AD patients, such as dermatitis on the face and extremities flexors[84]. The density of *S. aureus* population also effects the severity of AD[85].

The concept that *S. aureus* causes skin inflammation is supported by the fact that it colonizes even non-lesional skin of AD patients and induces dermatitis

similar to AD through *S. aureus* dysbiosis[81,86]. Additionally, modifications in the prevalent *S. aureus* microbiota during AD outbreaks imply that skin inflammation speeds up *S. aureus* colonization[83].

The exact correlation between *S. aureus* colonization and dermatitis-induced aggravation of AD is still unclear. Additionally, understanding the correlation between *S. aureus* colonization and AD exacerbation is difficult due to contradictory findings[83].

1.5.2 AD-specific *S. aureus* strains

These genetic findings suggest that the AD strain of bacteria colonizes the skin and creates an immune system specific to the AD pathogen[87].

The effects of skin diseases on *S. aureus* strains are important to note. In particular, the significance of strains isolated from severe AD patients has been noted. This is because *S. aureus* strains isolated from these patients caused more severe skin inflammation[87].

AD strains induce alterations in both Th1 and Th2 immune response adaptive processes (Figure 1.7). Additionally, pathological colonization of the skin by AD strains has not been studied. However, examination of lysosomes showed that AD strains specifically alter the morphology of keratinocytes. This led to the discovery that AD strains accumulate in lysosomes. When this happened, IL-1 production via TLR9 was found to be increased[88]. However, the effects of AD strain uptake into keratinocytes on skin pathology and inflammation remain undiscovered. In recent studies, cell-based complement activation helped maintain tissue stability by regulating the *Staphylococcus aureus* cell surface. These results suggest that AD patients depositing complement in their skin have irregularities in keratinocyte behavior[89].

The immune system response to the original strain of bacteria was distinct from the standard strain; this held true even when *S. aureus* stopped moving[90].

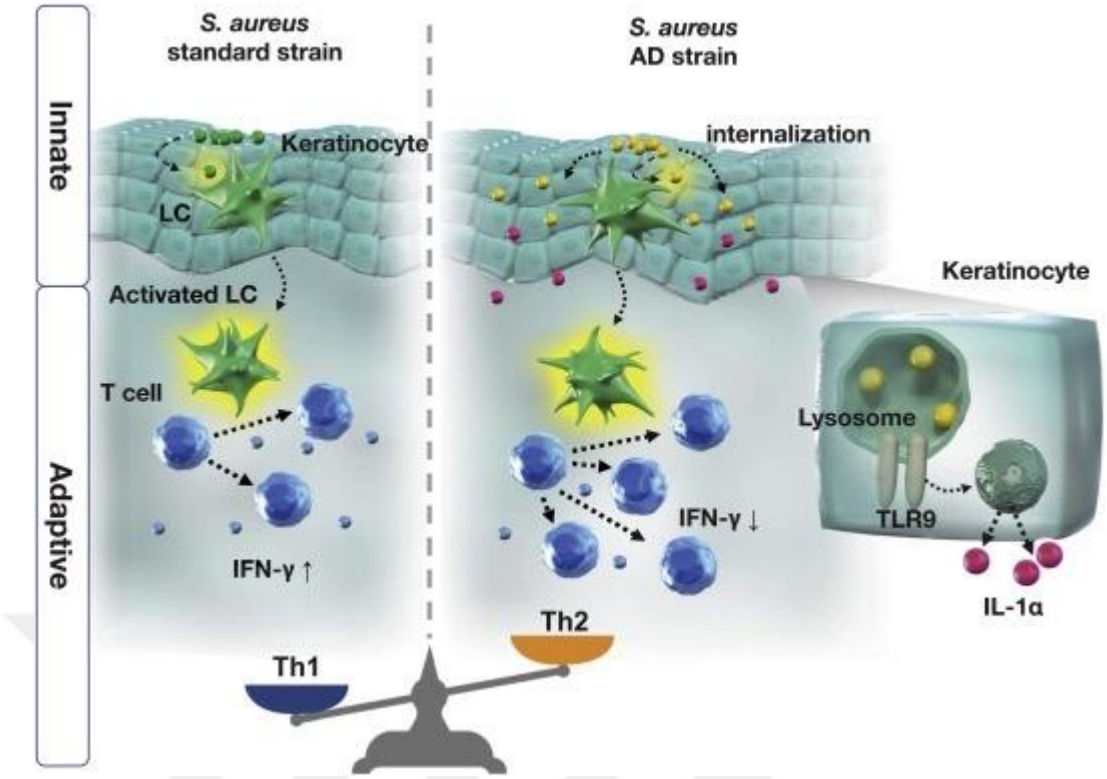

Figure 1.7 Compared the *S. aureus* in standard strain and in AD strain[87].

When compared to AD patients without *S. aureus* colonization, AD patients with *S. aureus* colonization had greater levels of Th2 biomarkers. Dupilumab, an inhibitor of IL-4 receptors, blocks subcutaneous dermal Th2 inflammatory response pathways and hinders *S. aureus* colonization. In the near future, brand-new systemic immunomodulatory biologics are anticipated. By modifying *S. aureus* cell wall proteins, it selectively targets skin immunity as well as *S. aureus* colonization[91].

1.5.3 *Staphylococcus aureus* and non-clinically infected eczema: colonizer or pathogen?

It is unclear whether *S. aureus* plays any role in eczema without an officially diagnosed infection. *S. aureus* can easily colonize the skin and grow in a favorable environment thanks to the clear advantages over the host. This is supported by the fact that treatment of eczema with topical steroids significantly reduced *S. aureus* colony counts[92].

S. aureus may additionally contribute to pathogenesis of eczema in numerous ways, including the manufacturing of several proteins such as

superantigens and proteases[93]. Superantigens penetrate the pores and skin barrier and cause chronic inflammation through several mechanisms, inclusive of:

- Stimulates T cells to release cytokines (proteins used in immune system signaling) [93].

- Allergen molecules act as allergens by triggering IgE antibodies that mast cells and basophils produce. This leads to inflammation and allergic reactions caused by mast cells and basophils[93].

- Inflammatory cytokines and keratinocytes, which are types of skin cells, are released by antigen-presenting cells and boosted by the increased T-cell infiltration caused by this stimulus[93].

- Inflammation increases because of increased skin homing receptors on T cells. This leads to increased migration of T cells to the skin and an increase in lymphocyte-associated antigen receptors[93].

- Increased skin irritation from other allergens is a side-effect of the allergic reaction[93].

1.6 Lipid profile

1.6.1 Total cholesterol (TC)

Cholesterol is a ring that contains oxygen, carbon and hydrogen side chains. It is easily soluble in oils and fats but nearly insoluble in water. Cholesterol can form esters with FA. Cholesteryl esters account for approximately 70% of cholesterol contained in lipoproteins. It is an essential part of cell membranes and prepares hormones and bile acids. It is produced by the adrenal glands, reproductive organs, intestine and liver[94].

1.6.2 Triglyceride (TG)

Triglycerides is esterified glycerol with three-chain fatty acids. These fats are the most common type found in a healthy human body, they are used to store energy and then used when needed. One way to add triglycerides during Lent is to add something like butter or oil[95].

1.6.3 High –density- lipoprotein (HDL)

Cholesterol is produced in the liver, but a critical role of HDL is to produce other lipoproteins. Enzymes called lecithin-cholesterol acyltransferase act in the liver to turn plasma cholesterol into cholesterol esters and cholesteryl esters that are then transported via transmembrane transport outside the liver. In addition to this, HDL is also where L-cholesteryl ester oxidase acts, this enzyme turns cholesteryl

ester into L-cholesterol. People with high HDL levels are healthier than those with low HDL levels[96].

1.6.4 Low density lipoprotein-cholesterol (LDL-C)

LDL refers to the result of VLDL being broken down by the lipoprotein lipase enzyme. It is referred to as “bad cholesterol” and the end product of VLDL degradation. The liver converts the larger IDL to LDL, this process removes more triglycerides. Additionally, LDL is formed and eliminated by the liver[97].

1.6.5 Association of serum lipid parameters with onset of atopic dermatitis disease

There are not many studies on the lipid levels of adults with atopic dermatitis. Some reports state that adults with atopic dermatitis have higher blood triglyceride levels, others claim these traits are unrelated to the condition[99].

Several studies examine the relationship between atopic Dermatitis and elevated lipids in adults and consistently shows adults with hyperlipidemia have more likely atopic dermatitis[100].

Studies in the past have shown dyslipidemia to be a chronic inflammatory state[101].

Increases in the level of LDL cholesterol and triglycerides increase the amount of pro-inflammatory signaling. This leads to increased levels of interleukin-6 and tumor necrosis factor-α in the blood. HDL cholesterol reduces inflammation by regulating T-cell activation. The presence of a chronic inflammatory disease like dyslipidemia can be the cause of chronic dermatitis. This supports the relationship between atopic dermatitis and high cholesterol levels, which can explain why many with atopic dermatitis also have elevated cholesterol[102,103].

2. AIM AND SCOPE OF THE STUDY

The purpose of this study was to extract the active substance from white mulberry and use for healing eczema disease for trying to decrease the uses of chemical drugs.

In this study we saw a good result of using it on patients and saw the positive changing in itching and irritation of the skin.

The extract was also tested on *S. aureus* bacteria after we discovered it on the patients with irritation on skin.

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemical Material

The major chemicals used in this study is provided in Table 3.1 and the instruments are listed in Table 3.2.

Table 3.1 List of chemicals and kits

Chemical substance	Company, Country
Dried mulberry (*Morus alba*)	Local store, Turkey
Ethanol 96% (C2H5OH)	Sasma BV, Netherlands
n-butanol (C4H10O)	Ravago, Belgium
NaCl	AP chemical, Singapore
Liquid Vaseline	Local store, Turkey
Sodium borate	Alibaba, China
White wax	Alibaba, China
Vit. E Kit	Mybiosource, USA
Granzyme B Kit	Mybiosource, USA
TC Kit	Mybiosource, USA
TG Kit	Mybiosource, USA
LDL Kit	Mybiosource, USA
HDL Kit	Mybiosource, USA

Table 3.2 List of instruments

Instruments	Company, Country
Oven	Cole-Parmer, USA
Reflux	Cole-Parmer, USA
Ultrasonic bath	Crest Ultrasonics, USA
Rotary evaporator	Cole-Parmer, USA
ELISA	Humareader, Germany
Spectrophotometer	Humareader, Germany
GC MASS	Agilent Technologies, USA

3.2 Methods

3.2.1 Extraction and purification

The sample (white mulberry) was collected from one of the bazaar in yalova city, 200g of dried mulberry fruit samples was collected, dried it more in a room temperature oven and then grinding it until obtaining fine powder, the fruit powder added into round bottom flask containing 1000 ml of ethanol 96%. The extraction was carried out at 60^{o}C for 24 hours using reflux setup, the extract was left for 1 day at room temperature shaking and for 2 days at room temperature steady, then

kept the flask in ultrasonic bath for 15 minutes. Filter solution using Whatmann No. 1 filter paper, and ethanol was evaporated at 50° C using Rotovap evaporator. The extract was dissolved in 100 ml of dH_2O, the material was transferred into a separation funnel and equal amount (100ml) of n-butanol was added. Two phases were separated for an hour. If more than two phases appeared in the funnel, we added 2 grams of NaCl, wait the mixture for another one hour again after shaking. We discarded the aqueous phase at the bottom, slowly and transfered the butanol phase into a round-bottom flask (Measured the weight of empty flask and recorded that as W_o). The butanol was evaporated at 80° C using rotary evaporator, it took around 30-45 minutes to obtain semi-dry extracts. The remaining butanol was removed under vacuum at room temperature for about an hour until obtaining dry extracts. Weight of the flask with dry extract (W_1) was measured, the extraction yield was calculated using following formula: ($W_1 - W_0$)\ 200. The extract was dissolved in dH_2O[104,105,106].

3.3 Preparation of ointment loaded with of mulberry fruit extract

3.3.1 Preparation of oil phase

Fourteen grams of white wax melted at 50° C, then stirred using a magnetic stirrer. When half of white wax was melted, liquid vaseline was mixed with melted wax and continued stirring at 70° C[107,104].

3.3.2 Preparation of aqueous phase

Sodium borate (0.25 g) was dissolved in dH_2O to prepare 4 ml solution at 70° C. Sodium borate was totally solubilized with 4ml extract solution[107,104].

3.3.3 Water-in-oil (W\O) emulsification

Droplets of aqueous phase were slowly added into oil phase. Magnetic stirrer was used to obtain the fine dispersion. When all aqueous phases were dispersed, an opaque semi-liquid mixture was obtained. The mixture was stirred till it cooled down to room temperature[107,104]:.

3.3.4 Biochemical analyze of mulberry extract in GC MASS device

A sample of white mulberry extract was taken to the Ministry of Industry and Minerals in Iraq to analyze the components of the extract. A 1µl of the sample was loaded in the GC MASS chromatography device (Figure 3.1), and 24 different elements were detected in the extract as shown in Table 3.6.

Figure 3.1 GC MASS device. Analytical Column: Agilent HP-5ms Ultra lent (30 m length x 250 µm diameter x 0.25 µm inside diameter), Injection volume 1µl and Pressure 11.933 psi; GC Inlet Line Temperature: 250 ˚C; Aux heaters Temperature 300 ˚C; Carrier Gas: He 99.99%; Injector Temperature: 250 ˚C Scan Range: m/z 25-1000; Injection Type: Split less; Oven Program: Temperature; Ramp 1: 60 ˚C hold to 3 min.; Ramp 2: 60 ˚C to 180 ˚C 7 ˚C/min; Ramp 3: 180˚C to 280˚C 8 ˚C/min; Ramp 4: 280˚C hold to 3 min

3.4 Clinical trials

This study included 110 individuals. They applied the ointment cream for 4 to 6 weeks. Swap sample was taken from site of eczema infection for microorganism investigation before ointment application, the individual divided into three groups:

-Group one: 38 patients of eczema use the ointment for 4 to 6 weeks, most of the patients have various health conditions such as high blood pressure, diabetes and high cholesterol.

- Group two: 37 individuals without eczema use the ointment for 4 to 6 weeks.

- Group three: 35 individuals of eczema use a cream doesn't contain any active substance.

3.5 Blood Sample collection

The collection of samples included 10ml of venous blood, placed in the tube at room temperature and centrifugated for 5 minutes at 3,000 cycles/min. The serum was put into an Eppendorf tube and stored in -20º C freezer until it was analyzed. The blood samples collected before using the cream by the patient and after 4 to 6 weeks of using the cream to analyze (Granzyme B, Vit. E) by ELISA and (TC, TG, HDL, LDL) by Spectrophotometer.

3.6 The antibacterial activity of the extract

The antibacterial activity of the extract was determined against *S. aureus* which was shown to be associated with my eczema patients, using paper disc diffusion method. Overnight growth (24h) culture of the bacterium were adjusted to ($1x10^8$ UFC/ml) equivalent to (OD= 0.5 on McFarland) was spread on surface of sterile nutrient agar. Six millimeters diameter of Whatman filter paper discs (GF/C) were sterilized in a petri dish at 121°C for 15 min. after sterilization, each disc was immersed with 100µl of different of the extract (1.5 to 3 %) . Distilled water was used as a control because it has no antimicrobial activity. Then plates incubated at 37°C for 24h[108]. Following the incubation the inhibition zone diameter was measured.

4. RESULTS

4.1 Biochemical properties of the mulberry extract by GC MASS

Biochemical content of mulberry extract were tested by GC MASS device at the Ministry of Industry and Mineral Resources. The chemicals qualified from the extract were listed in Table 4.1. The peaks of the extract were shown in Figure 4.1.

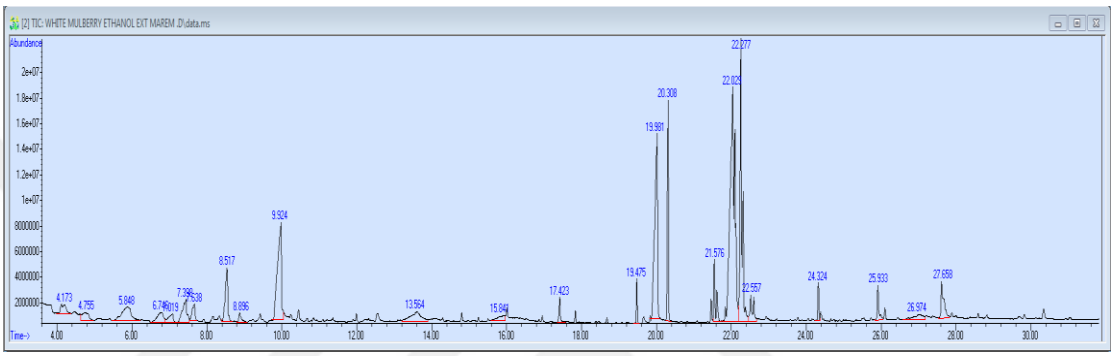

Figure 4.1 GC MASS for mulberry extract analyzes.

Table 4.1 Description of Peaks and compound for GC MASS mulberry extract analyzes.

Peak number	Compound Name	Retention time	Percent Distributions
1	1-Butanol	4.173	1.142 %
2	Piracetam	4.755	1.233 %
3	Cyclopentanol	5.848	3.276 %
4	Piperazine	6.746	1.562 %
5	Pentanoic acid	7.019	0.998 %
6	Cyclooctanecarboxylic acid	7.398	2.490 %
7	Butanoic acid	7.638	1.210 %
8	1,3-Dioxane, 4-methyl uran-3-one	8.517	3.280 %
9	N-(n-Propyl)acetamide	8.896	0.614 %

10	5-Hydroxymethylfurfural	9.924	9.375 %
11	1,4-Dioxane-2,6-dimethanol	13.564	2.528 %
12	Urethane	15.841	0.968 %
13	Tetradecanoic acid	17.423	0.825 %
14	Hexadecanoic acid	19.475	1.024 %
15	n-Hexadecanoic acid	19.981	12.205 %
16	Hexadecanoic acid, ethyl ester	20.308	5.805 %
17	11-Octadecenoic acid methyl ester	21.576	2.648 %
18	9-Octadecyne	22.029	27.289 %
19	Linoleic acid ethyl ester	22.277	13.667 %
20	9,12-Octadecadienoic acid	22.557	1.820 %
21	Isopropyl linoleate	24.324	0.985 %
22	Glycerol 1-palmitate	25.933	1.403 %
23	Vitamin E	26.974	1.199 %
24	7-Hexadecyn-1-ol	27.658	2.453 %

In the previous study which was about GC-MS fingerprints combined with chemometric analysis for the authentication of *Morus alba* leaves, several compounds were common compared to our results[114].

Many of these compounds use in skin and beauty products or in medicines:

- Piracetam use as dietary supplement[115].

- Piperazine is mainly used as a buffering agent in any formulation. It provides with neutral to basic pH (7.0-7.6 pH) in any formulation. It is mainly used in conjunction with acids, which can make the product unstable otherwise. When a buffer is added in any formulation, it helps maintain the quality of any product for a longer time. After topical application, the epidermal layer of skin gets an enzymatic treatment, which helps the dead skin cells to loosen and come off easily. Exfoliation helps get rid of dirt microbes as well as dead skin cells, making skin look eve-toned and youthful[116].

- Benefits of benzoic acid in skincare include anti-aging, soothing, and moisturizing properties. In addition, a major derivative of benzoic acid, known as

phenolic veratric acid, contains high concentrations of antioxidants to help neutralize free-radicals present in environment[117].

- Butanoic acid, It has been discovered that butyrate increases antioxidant levels, lowering the risk of developing inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS)[118].

- Urethane forms a protective film on the skin, which enhances water resistance and prolongs the efficacy of the products. It also improves the texture of the formulations, providing a smooth and silky feel to the skin[119].

- Octadecenoic acid is used as a surfactant and emulsifying. agent for fragrance and as the base for other fatty acid ingredients that are synthesized into emollients and lubricants it is also used in shampoos, shaving creams, and detergents[120].

4.2 Impact of *Morus alba* extract on eczema

In this study we have a total of 110 individuals that includes patients 73 and healthy person 37, the individuals who used the ointment were of different ages, genders, and medical conditions.

The levels of (Vit.E , Granzyme B , TC , TG , LDL , HDL) in blood serum samples were studied for every patients and healthy person.

We also tested the potential antimicrobial effect of the extract from the white on the *S. aureus.*

4.2.1 Group one

The first group contains 38 patients with eczema who tried the ointment for a period of 4 to 6 weeks and the results for the analyzes were good and we notice an improvement, albeit a slight one, in all the analyzes based on the existing chart. Our data showed that the control was constant for all the three groups. We notice the change in the conditions (itchy, irritation) before treatment and after treatment (Figure 4.2).

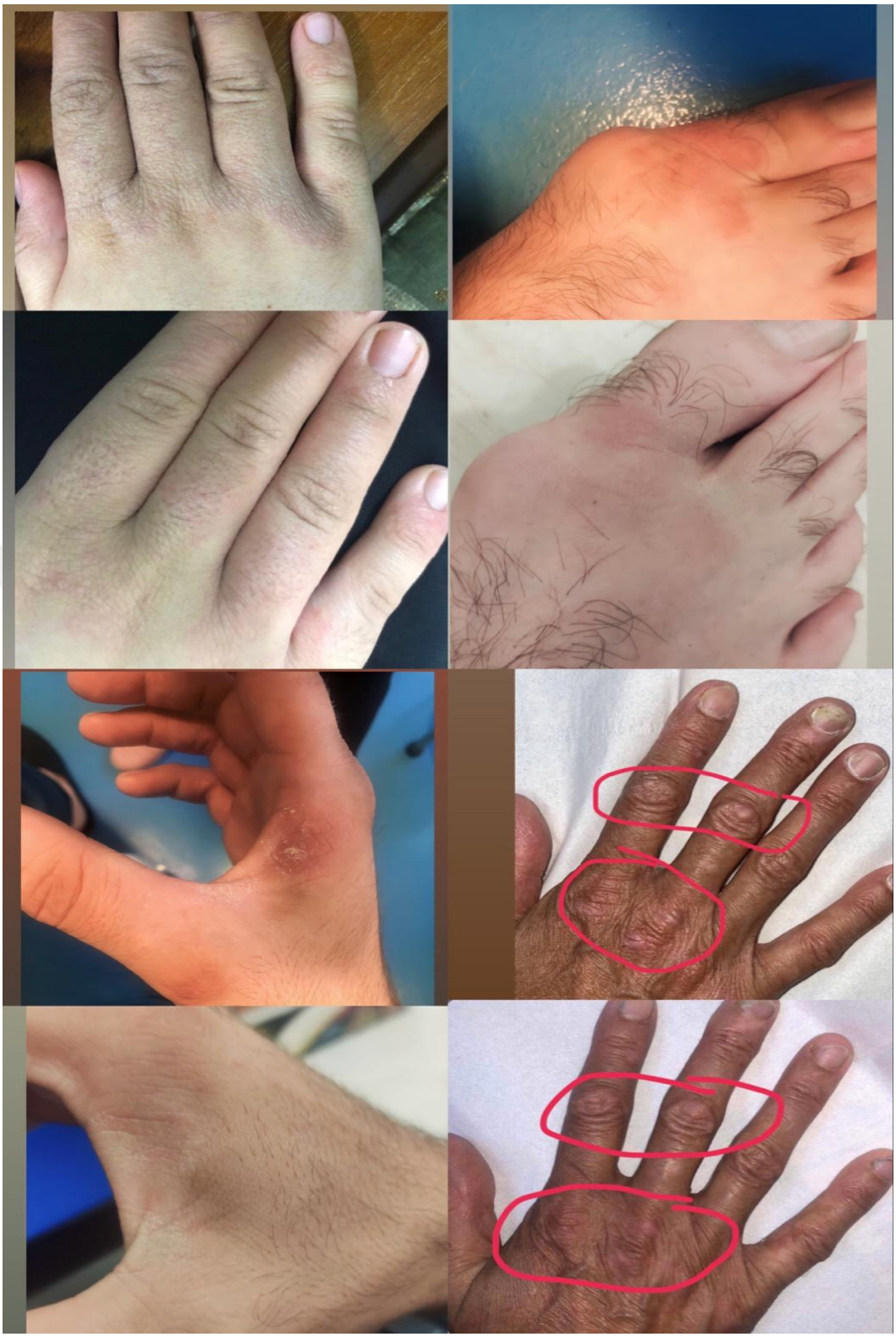

Figure 4.2 Result for our patients before using the ointment and after using it in period 2 to 4 weeks. (Taken by Maryam Sabeeh)

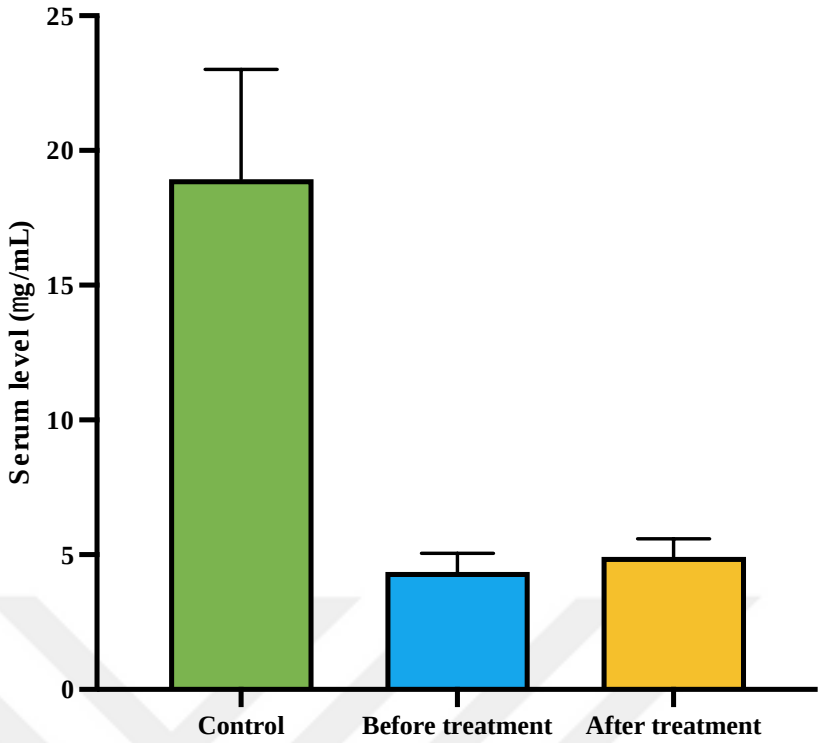

Figure 4.3 The mean/graph average for changing in vitamin E in AD patients for group one.

We can see for the result of Vitamin E after using the ointment in period 4 to 6 weeks there is increasing in the numbers of the vit.E for AD patients (Figure 4.3). In another clinical study on 96 patients with AD tried oral vitamin E (400 IE/day) for 8 months, vitamin E decreases serum levels of IgE in AD patients. The relation between intake vitamin E, IgE levels, and the clinical symptoms of atopic dermatitis that vitamin E could be a therapeutic medicine[110].

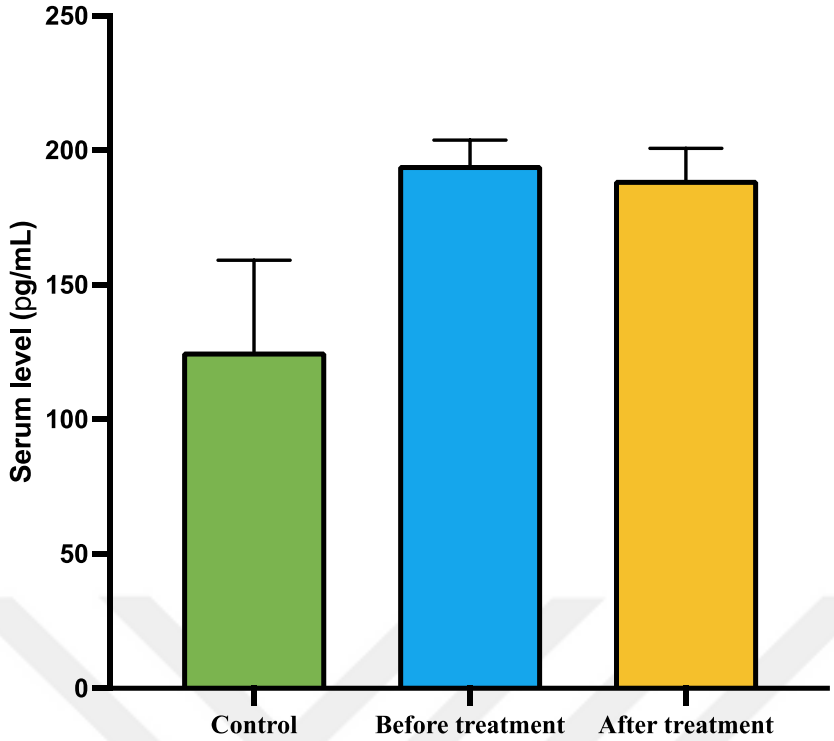

Figure 4.4 The mean/graph average for changing in granzyme B in AD patients for group one.

In this study we can see an improvement in Gr.B levels for the AD patients after using the ointment in around 4 to 6 weeks (Figure 4.4).

In another study for the relationship between Gr.B and lung cancer, the findings suggested that the predictive power of Gr. B for lung cancer detection was even superior. The combination of Gr.B can enhance the process of choosing of patients with high-risk for lung cancer[111].

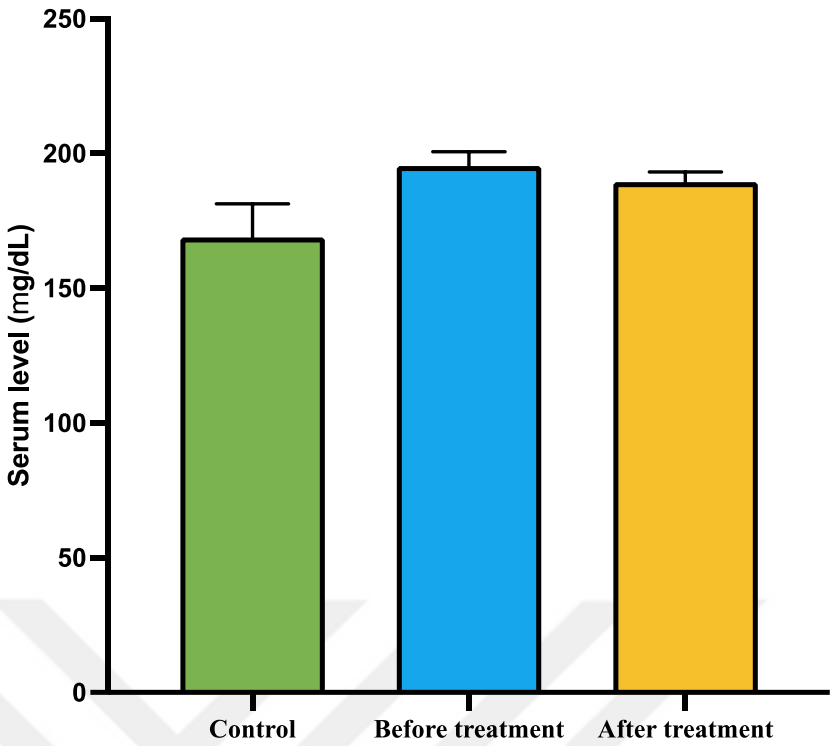

Figure 4.5 The mean/graph average for changing in Total Cholesterol in AD patients for group one.

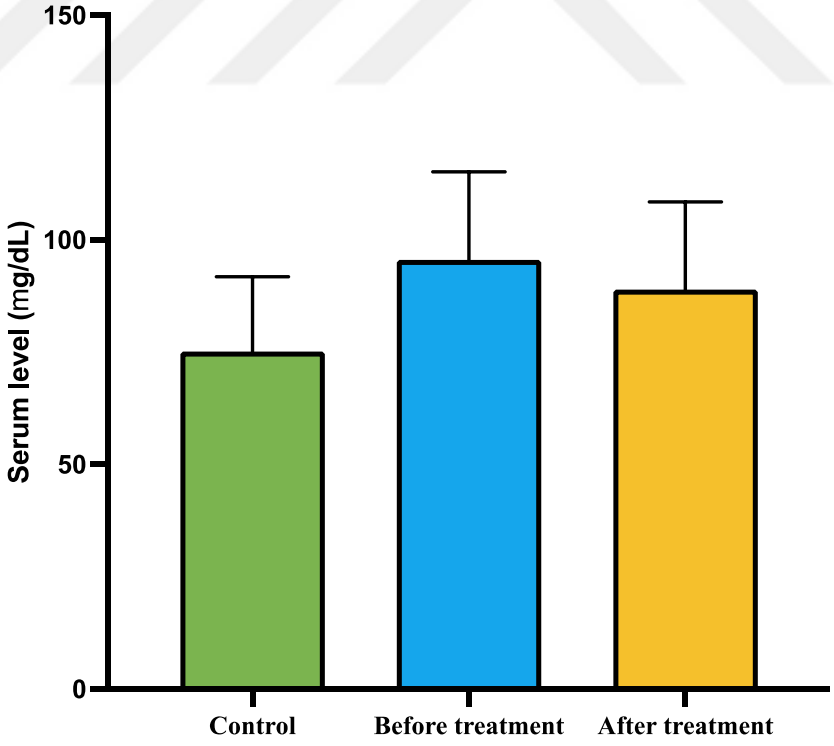

Figure 4.6 The mean/graph average for changing in triglyceride in AD patients for group one.

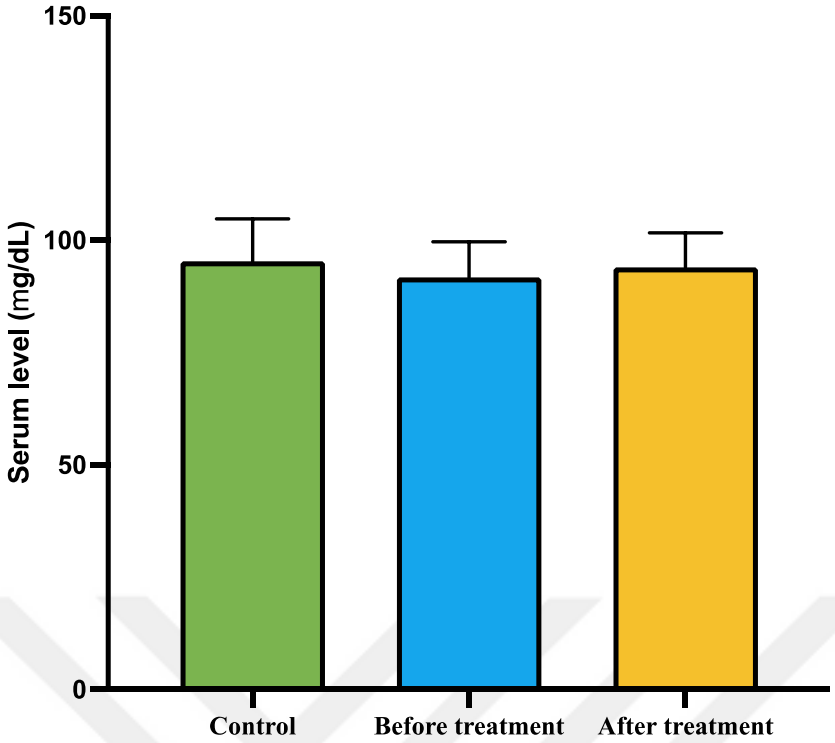

Figure 4.7 The mean/graph average for changing in low density lipoprotein in AD patients for group one.

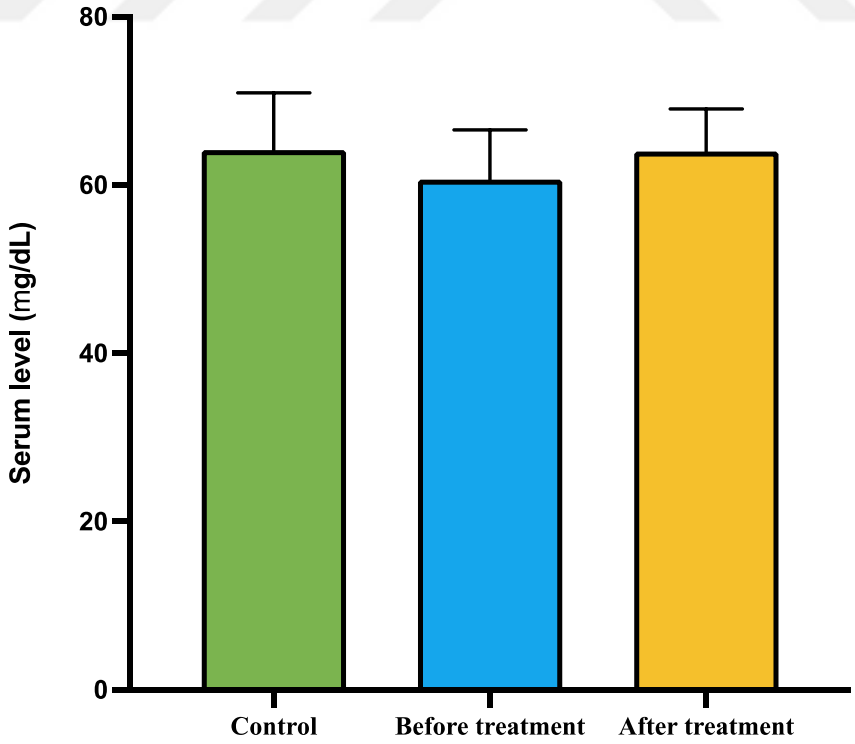

Figure 4.8 The mean/graph average for changing in high density lipoprotein in AD patients.

For the result of lipid profile in AD patients we note a slight change in the numbers of blood serum analyzed (Figures 4.5, 4.6, 4.7, 4.8).

For another study to J Clin Biochem Nutr, 2010 who tried an oral application for 12 weeks rich in mulberry leaf extraction and 1-Deoxynojirimycin to see the effect on lipid profile in human[109].

Table 4.2 The demographic distribution data for before and after treatments of the Group one.

Group one	Mean ± SEM	Significance
Vit. E (µg/ml)	4.56551 ± .89047	.002
Granzyme B (pg/ml)	191.72451 ± 10.844931	.021
TC (mg/dl)	192.39121 ± 5.435245	.000
TG (mg/dl)	92.25703 ± 19.712054	.107
LDL (mg/dl)	61.73303 ± 8.311257	.010
HDL (mg/dl)	92.8747 ± 7.82435	.207

We note that (Vit E, granzyme B, TC, LDL) there is a moral difference, meaning that receiving the treatment affected the results of the analyzes, but there is no significant difference before treatment and after treatment in the analyzes (TG, HDL), meaning that the treatment did not affects the change of analysis results (Table 4.2). Tthese results can also be seen through the ROC curve Figure 4.8.

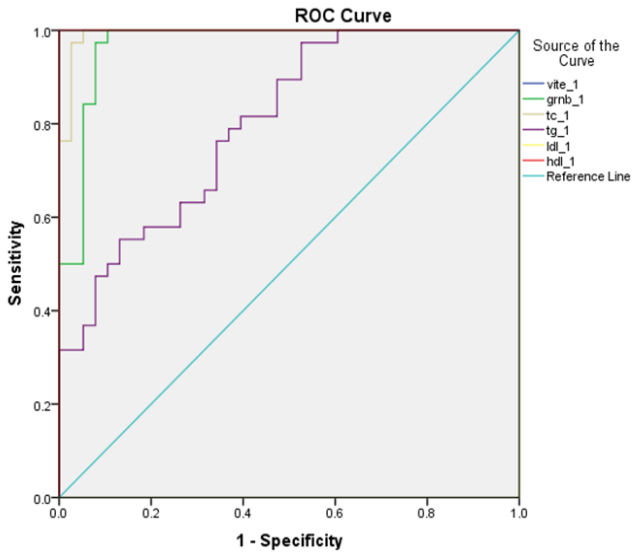

Figure 4.9 ROC Curve for group one

4.2.2 Group two

It contains 37 healthy people without eczema who tried the ointment for a period of 4 to 6 weeks.

When installing the control for all the analyzes, we notice that the values of all the analyzes were not affected or changed. This means that the treatment did not affect the values of the analyzes, and also, the skin of a healthy person is not allergic to the ointment.

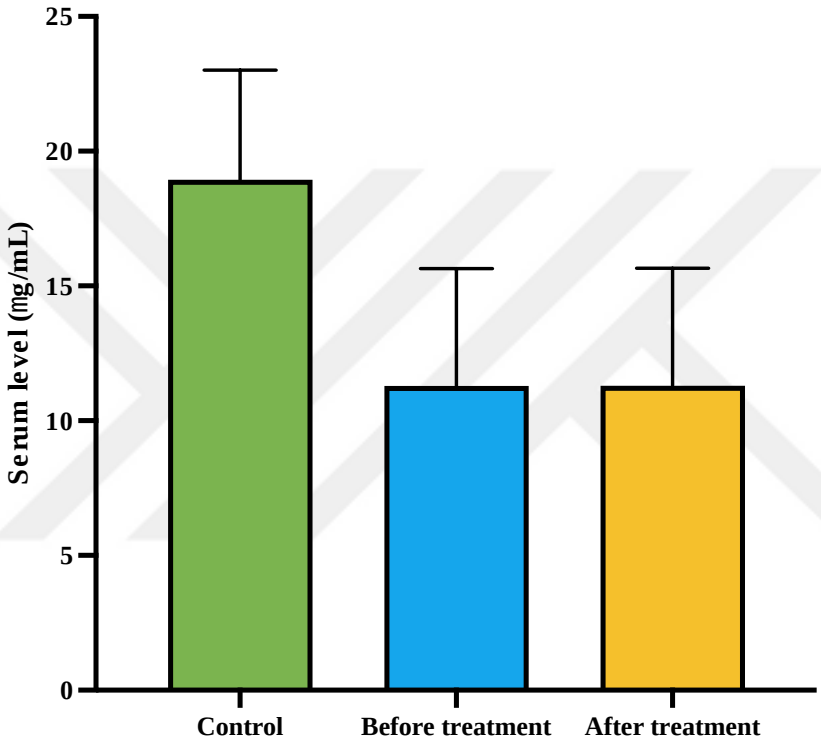

Figure 4.10 The mean/graph average for changing in vitamin E in healthy people tried the ointment for group two.

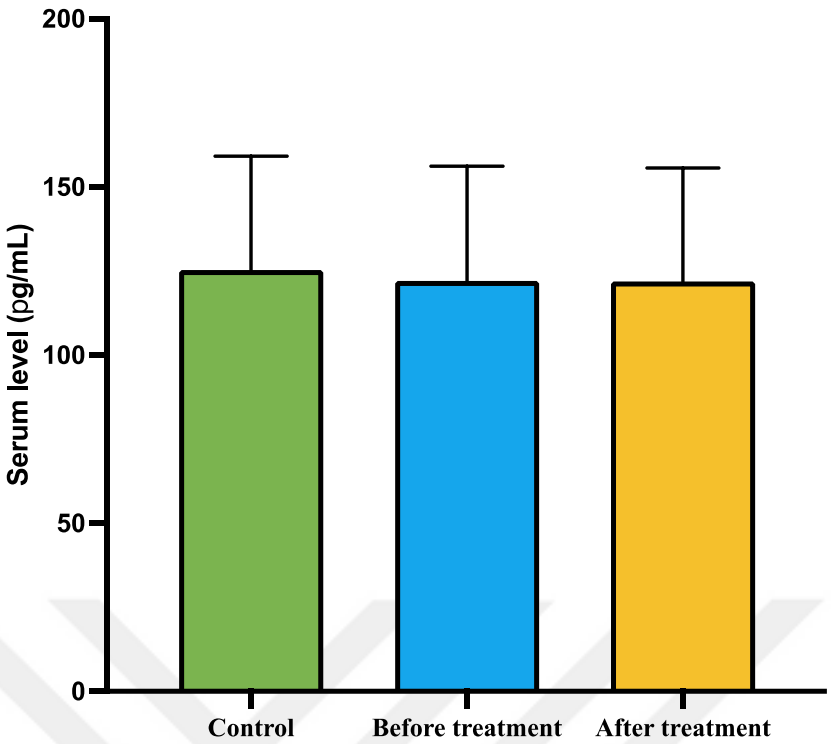

Figure 4.11 The mean/graph average for changing in granzyme B in healthy people tried the ointment for group two.

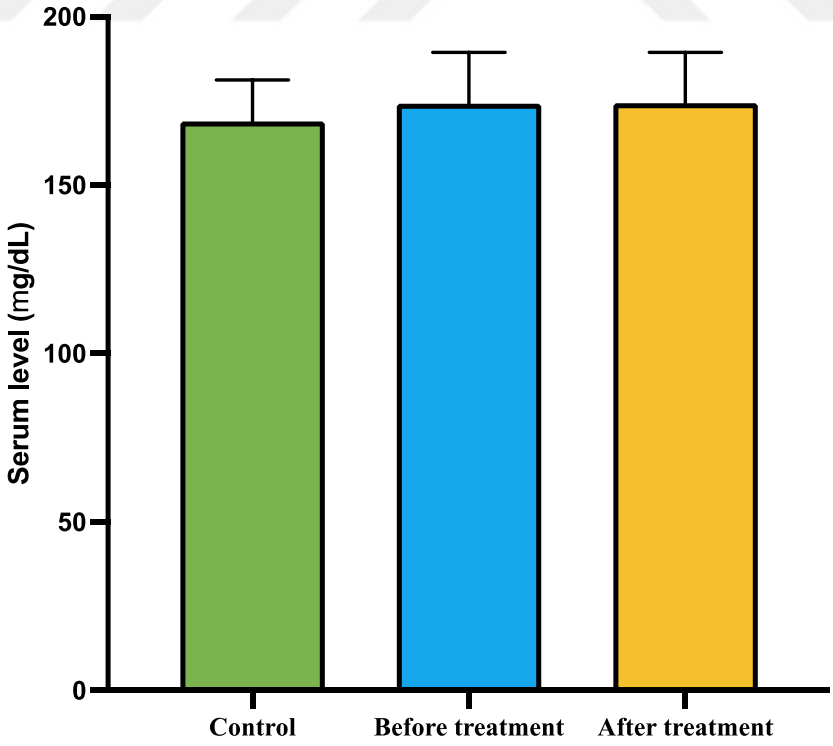

Figure 4.12 The mean/graph average for changing in total cholesterol in healthy people tried the ointment for group two.

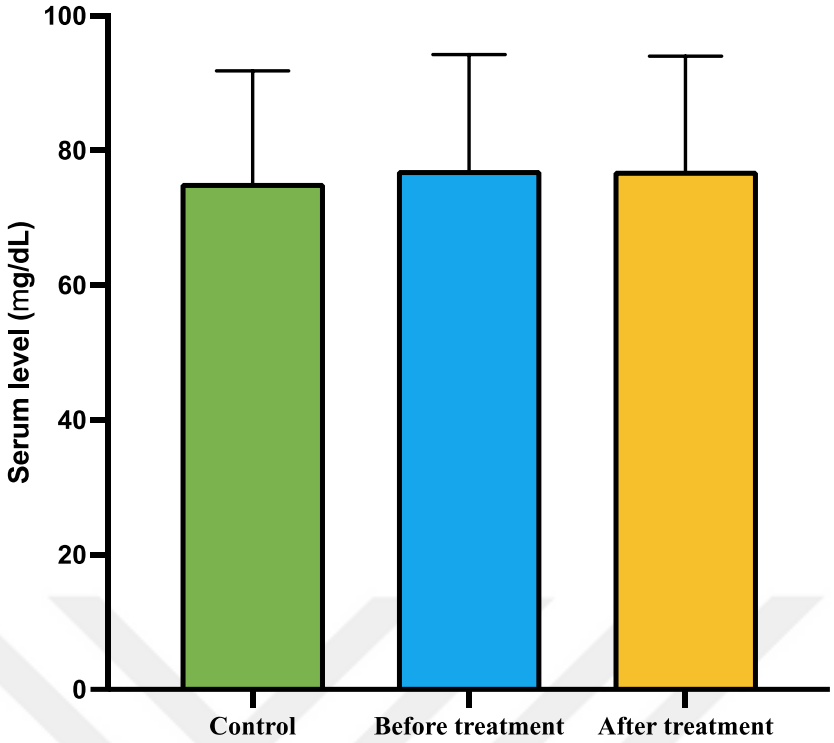

Figure 4.13 The mean/graph average for changing in triglyceride in healthy people tried the ointment for group two.

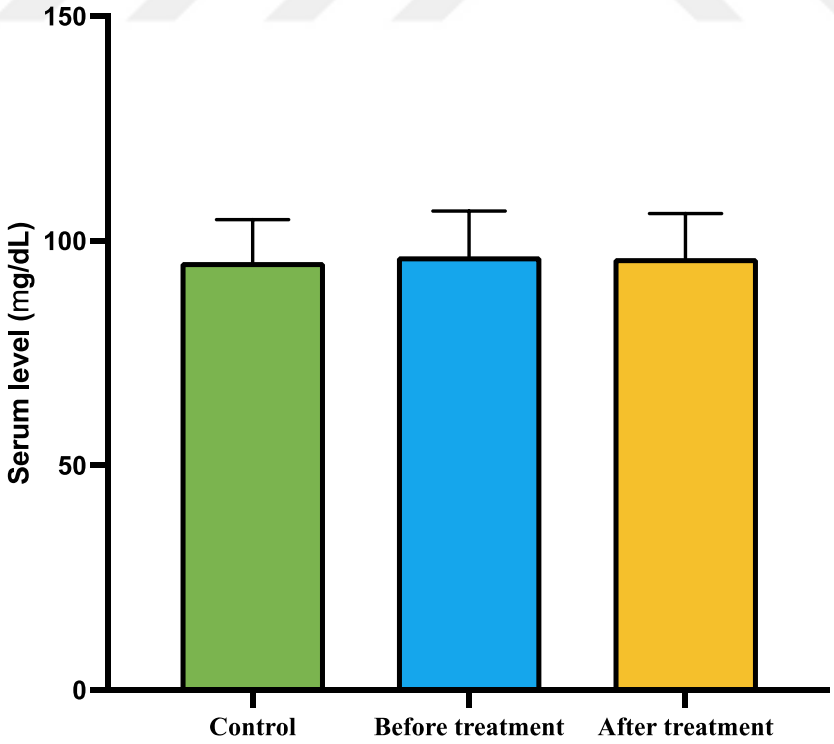

Figure 4.14 The mean/graph average for changing in low density lipoprotein in healthy people tried the ointment for group two.

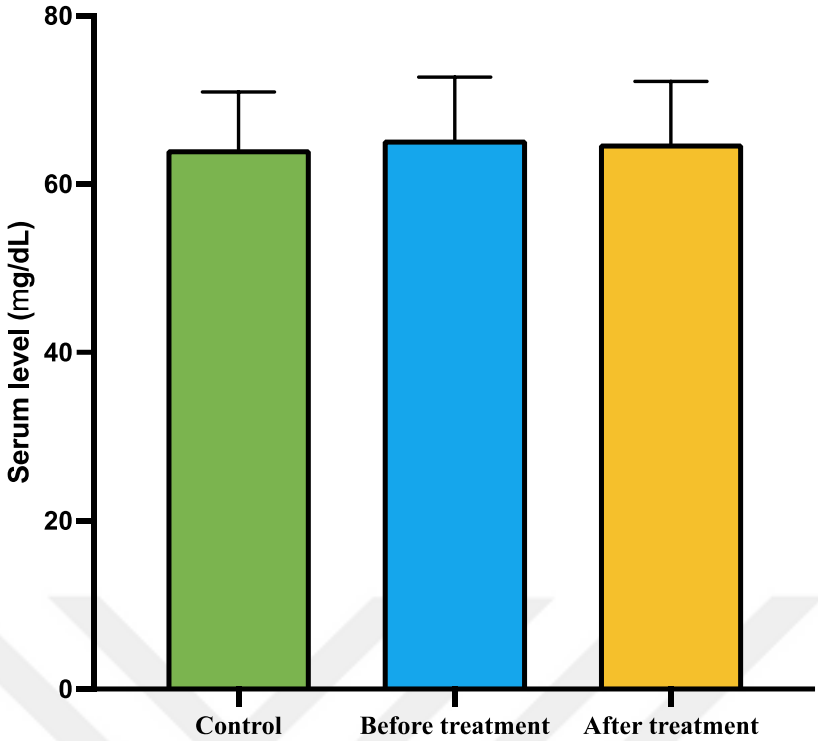

Figure 4.15 The mean/graph average for changing in high density lipoprotein in healthy people tried the ointment for group two.

There is no significant difference before treatment and after treatment in all analyzes (Table 4.3), meaning that the treatment did not affect the change in the results of the analyzes (Figures 4.10-15).

Table 4.3 The demographic distribution data for before and after treatments of Group two.

Group two	Mean ± SEM	Significance
Vit E (µg/ml)	11.29377 ± 4.328401	.922
Granzyme B (pg/ml)	121.90345 ± 33.710751	.966
TC (mg/dl)	174.27661 ± 15.04029	.880
TG (mg/dl)	77.06489 ± 16.938691	.944
LDL (mg/dl)	96.43223 ± 9.878254	.833
HDL (mg/dl)	65.06364 ± 7.336645	.665

These results are provided as the ROC curve, as shown in Figure 4.16.

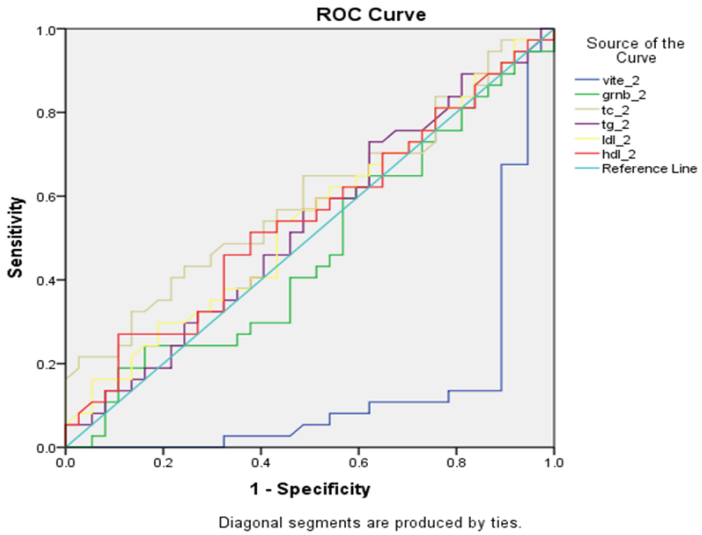

Figure 4.16 ROC Curve for group two.

4.2.3 Group three

It contains 35 patients with eczema who tried an ointment that does not contain a therapeutic substance or an extract. Through the drawings, we notice a slight change in the results of the analyzes. This may be due to the reason that they did not use a treatment for eczema for a period of 4 to 6 weeks, which leads to increased inflammation and irritation.

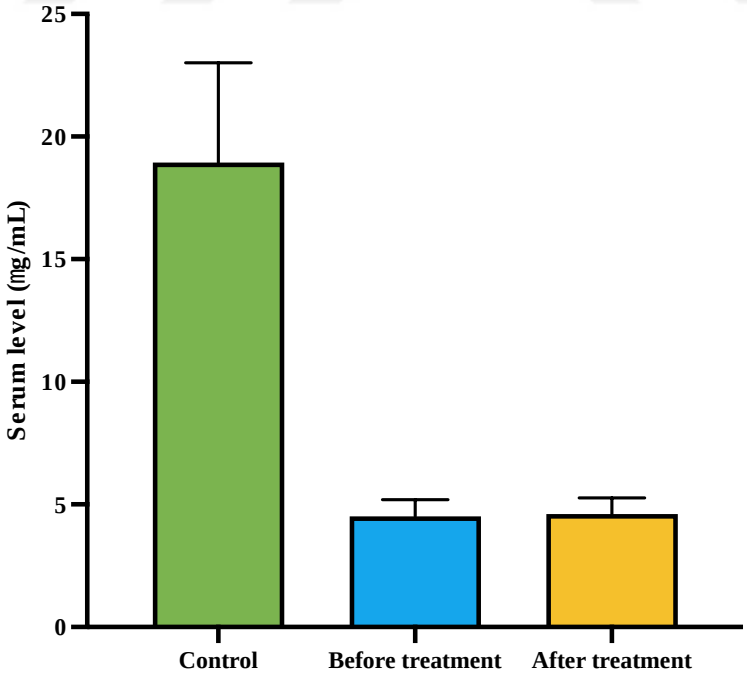

Figure 4.17 The mean/graph average for changing in vitamin E in patients with eczema tried the ointment does not contain extract for group three.

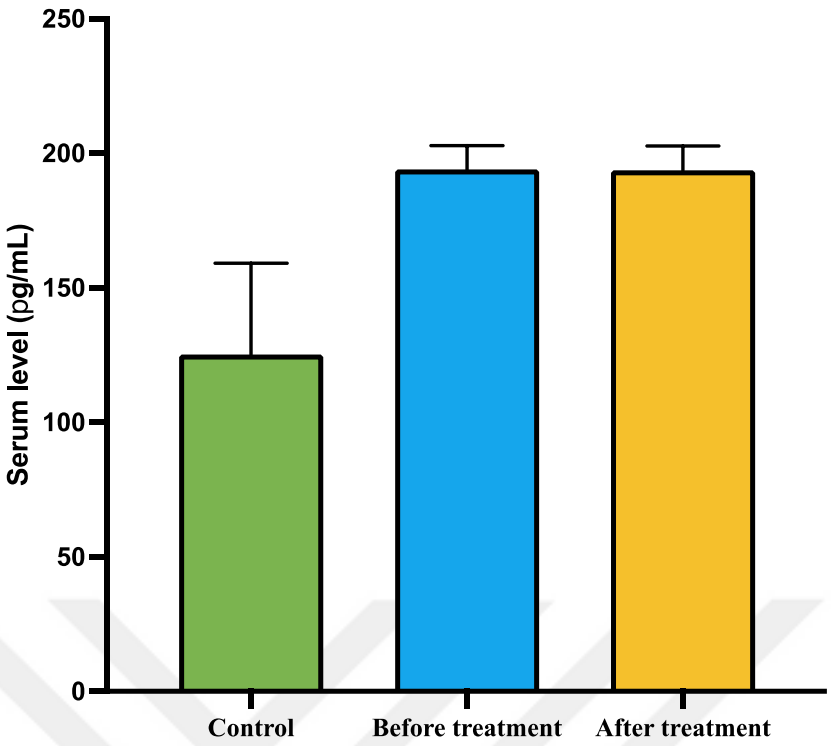

Figure 4.18 The mean/graph average for changing in granzyme B in patients with eczema tried the ointment does not contain extract for group three.

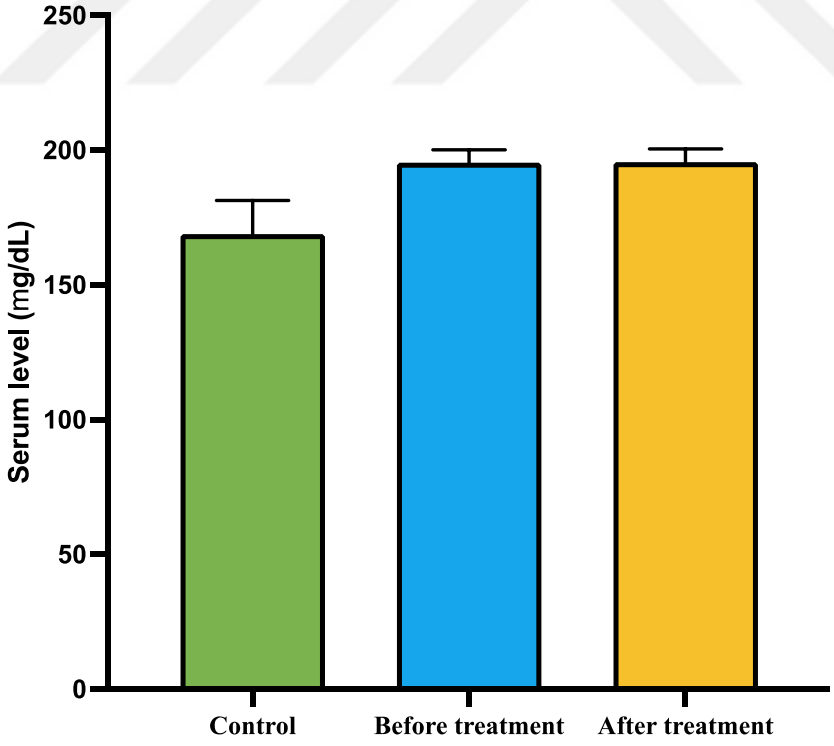

Figure 4.19 The mean/graph average for changing in total cholesterol in patients with eczema tried the ointment does not contain extract for group three.

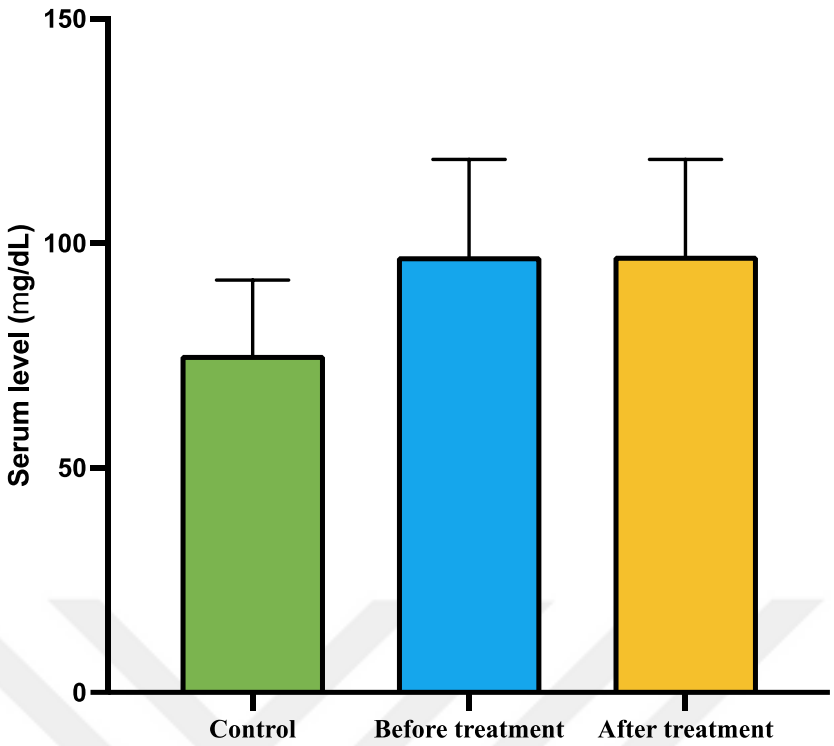

Figure 4.20 The mean/graph average for changing in triglyceride in patients with eczema tried the ointment does not contain extract for group three.

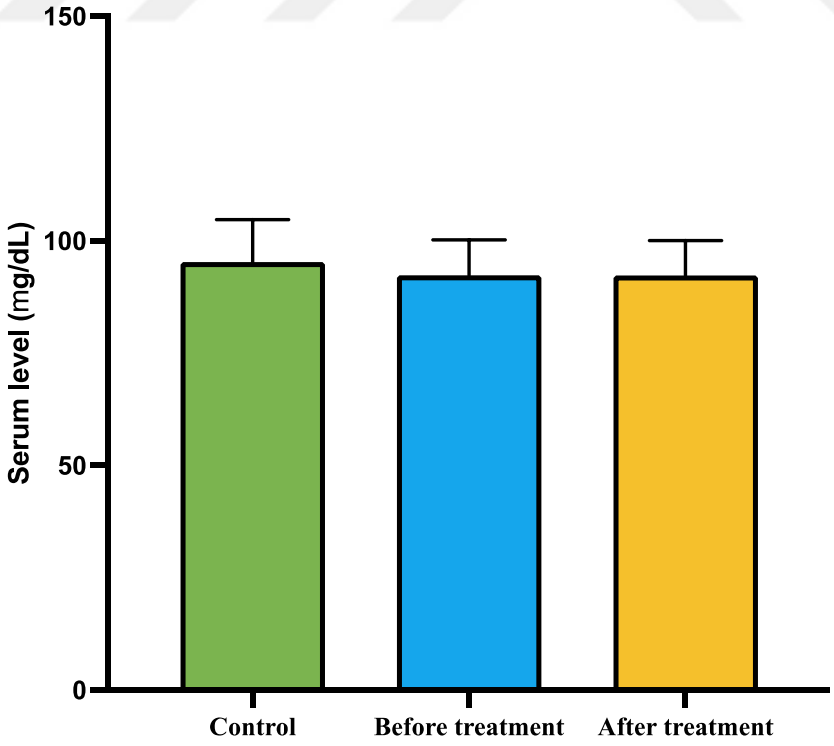

Figure 4.21 The mean/graph average for changing in low density lipoprotein in patients with eczema tried the ointment does not contain extract for group three.

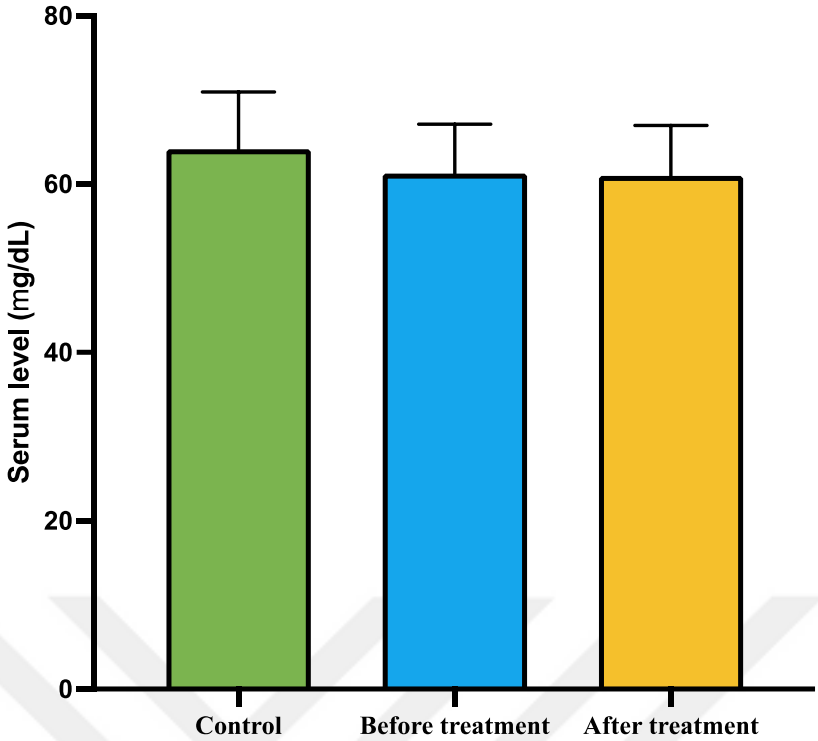

Figure 4.22 The mean/graph average for changing in high density lipoprotein in patients with eczema tried the ointment does not contain extract for group three.

Table 4.4 The demographic distribution data for before and after treatments of the Group three patients.

Group three	Mean ± SEM	Significance
Vit E (µg/ml)	11.61254 ± 7.740536	.000
Granzyme B (pg/ml)	159.20053 ± 43.225651	.000
TC (mg/dl)	181.43267 ± 16.628334	.000
TG (mg/dl)	86.80429 ± 21.752271	.000
LDL (mg/dl)	93.48270 ± 8.625103	.485
HDL (mg/dl)	62.78834 ± 6.540725	.055

Our results showed a significant difference for the analysis of Vit E (Figure 4.17) Granzyme B (Figure 4.18), TC (Figure 4.19) and TG (Figure 4.20). However there was no significant difference for the analyzes HDL (Figure 4.21), LDL (Figure 4.22) between before treatment and after treatment (Table 4.4 and Figure 4.23).

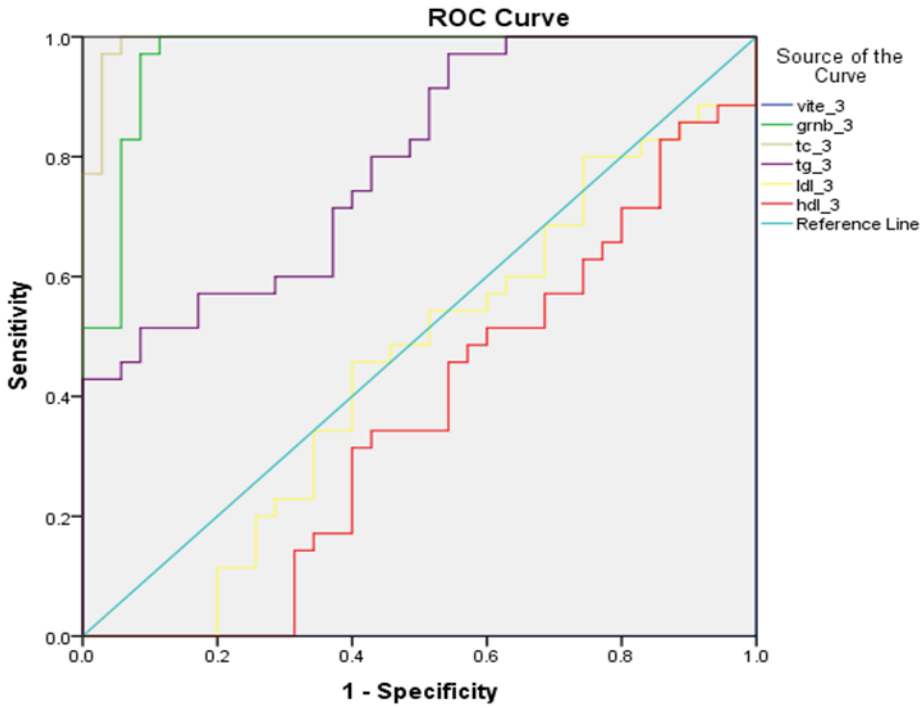

Figure 4.23 ROC Curve for group three

4.3 Antibacterial Activity of the Extract

The antibacterial activity of the extract against *S. aureus* was analyzed. The extract was effective against *S. aureus* at concentrations 1 to 3 % and showed total inhibition 9 to 16 mm , as showed in Table 4.5 and Figure 4.24.

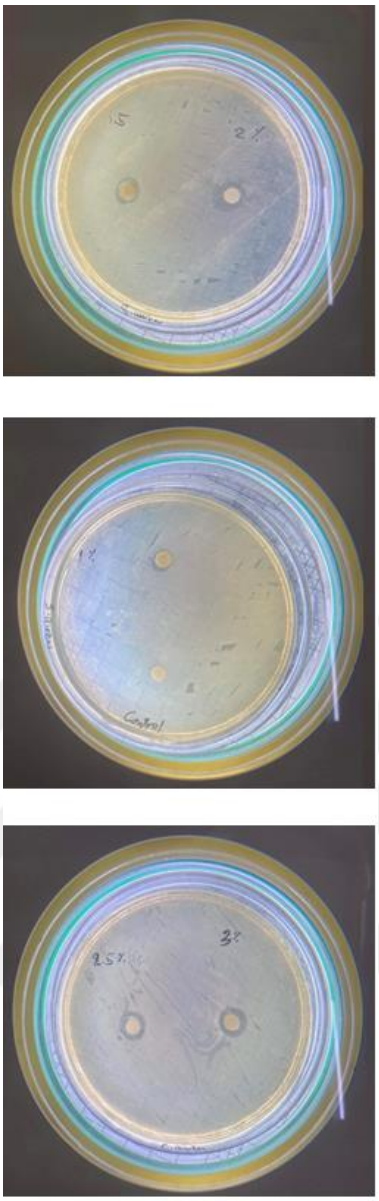

Figure 4.24 Antibacterial activity of extract against *S. aureus*, control size of inhibition zone (zero). (Taken by Maryam Sabeeh)

Table 4.5 Zone of inhibition by the extract

Concentration of the extract	Diameter of inhibition zone (mm)
1 %	9 mm
1.5 %	12 mm
2 %	16 mm
2.5 %	14 mm
3 %	14 mm

5. CONCLUSIONS AND RECOMMENDATIONS

In this research, and because of the experiments conducted within our study, we notice that there are statistical differences between the groups and between the same group and the control group.

The first group, which contains 38 patients with eczema. It was noted that there was a slight improvement in the Vit. E analysis. As for the Granzyme B analysis (atopic dermatitis), it is normal to be high or very high in patients. When using the treatment, it was noted that there was a slight to good response to the treatment due to the low level of ulceration and irritation in patients. Good improvement and slight changes were found in the lipid profile analysis of the patients after treatment.

The second group, which contains 37 of healthy people. We note that there is no change or differences in all the analysis, or the presence of any sensitivity on the skin of healthy people.

The third group, which contains 35 patients with eczema, applied an extract free ointment. We observed that there is no changes in the results of the analysis, while it remains within the abnormal level.

From the above results we conclude:

- The ointment is effective for treating eczema.

- The ointment is efficient for reducing skin ulceration caused by eczema.

- The ointment is functional as an antibiotic as a result of its experiment on bacteria (*S. aureus*).

- When analyzed the extract by GC MASS device we notes that the extract contain 24 elements and the main compounds used as dietary supplements or in skin products as well as the extract contain vitamin E the important element that use for healing eczema.

6. RECOMMENDATIONS

- In this study, we use 0.5% of mulberry extract in the ointment for period 4 to 6 weeks. We recommend other researchers to use different concentrations of the extract and extra period of trial on patients.

- Combining different extracts of vitamin E fruits to enhance the activity of the ointment.

-Showing the antibacterial activity of the extract on other type of pathogenic bacteria with different concentrations, learning MIC and MBC of the extract.

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8. APPENDICES

Appendix 1. The scanned copy of ethical approval document

University of Baghdad
College of Science for women
Research ethics committee

Application for Biomedical Research Ethics Review

PART 1: IDENTIFICATION		
1.1	**Project Title** Evaluation of biochemical properties of Morus alba fruit	
1.2	**Principal Investigator** Department: Chemisitry	
	If this is a student/graduate/resident project, please provide the following information:	
	a) Student Name: Maryam Sabeeh Madhloom	b) Supervisors Name: Ercan Selçuk Ünlü, and Ekhlass M. Taha
1.3	**Research Site(s) where project will be carried out:** University of Baghdad	
1.4	Proposed Project Period: F	
1.5	**Do you consider this project to involve?** ☐ Minimal Risk ☐ More than Minimal Risk ☒ No Risk	
1.6	**Name of funding source:** No funding source	

PART 2: REGULATORY REQUIREMENTS	
2.1	**The project involves intervention study (Clinical trials):** ☒ YES ☐ NO **If the answer is YES, proceed to 2.2; otherwise go to part 3**
2.2	**Clinical trials are required to be registered with https://www.who.int/clinical-trials-registry-platform (or with official platform where the research is conducted).** Ethical permission for this study was obtained from the Research Ethics committee of scientific of college of science for women department of chemistry with session 14, number 387 at 19/6/2022

PART 3: BRIEF OVERVIEW OF RESEARCH PROJECT (two page maximum)	
3.1	**Research Question/ Hypothesis** Is Morus alba fruit extract effect on patients with eczema?
3.2	Research Design/Methods Extraction of Morus alba fruit active compound. Evaluation of the biochemical properties Study it is effects on some biochemical parameters

PART 4: PARTICIPANT RECRUITMENT	
4.1	**How many participants will be enrolled in the project:** Globally? Locally? 60
4.2	**Provide a detailed description of the method of recruitment.** a) How will prospective participants be identified? physician b) Who will contact prospective participants? **researchers** c) How will this be done? phone and during routine visiting physician

PART 5: CONSENT PROCESS	
5.1	**Describe the consent process**: a) Who will ask for consent ? researchers b) Where, and under what circumstances? during routine visiting physician
5.2	**How long will the participant have to decide whether or not to participate?** If less than twenty-four hours, provide an explanation. **At any time**
5.3	**Will all participants be able to consent on their own behalf?** ☒ YES ☐ NO If **No**, explain why: a) If a participant is unable to consent, who will consent on his/her behalf? Parents only **b)** Will the participant be able to assent to participate? ☐ YES ☒ NO
5.4	**If monetary compensation or reimbursements for expenses will be offered to the participants, please provide the details.** There are no risk so, there is no reimbursements offered to participants.
5.5	**Describe your plans for providing project results to the participant? Contact them with phone**

PART 6: PROCEDURES AND RISKS	
6.1	**What are the known risks associated with the project procedures?** NO Risks
6.2	**What strategies will be put in place to minimize and/or manage the potential risk(s) to participants and other affected individuals?**

PART 7: DATA SECURITY AND STORAGE

7.1	**Indicate from which sources personal and health information data will be collected:** ☒ Participant data collected prospectively for the purpose of this project ☐ Ministry of Health ☐ Other – please specify: ☐ Not applicable (No personal or health information to be collected). Proceed to Section 8.
7.2	**How will the confidentiality of participants and their health information be protected?** The researchers will use code for each participant, and no one allow to use the researcher's computer.
7.3	**Describe the storage arrangements and final disposition of the project data collected.** The project data will storage in researcher's computer.
7.4	**List the project personnel who have access to any identifiable personal health information and who will have access to any list that links participant names to their project ID number, consent form, enrolment log, etc. Researchers only**

PART 8: CONFLICT OF INTEREST

8.0	**Is there any real or perceived conflict of interest (any personal or financial interest in the conduct or outcome of this project)? Will any of the researcher(s), members of the research team and/or their immediate family members:** ☐ YES ☒ NO If yes, please describe the personal benefits or relationship.

PART 9: DECLARATION BY PRINCIPAL INVESTIGATOR *(OR SUPERVISOR FOR STUDENT PROJECTS)*

Project Title:

Evaluation of the biochemical properties of Morus alba fruit extract and its healing effect on patients with eczema

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- I confirm that the information provided in this application is complete and correct.
- I accept responsibility for the ethical conduct of this project and for the protection of the rights and welfare of the human participants who are directly or indirectly involved in this project.
- I will ensure that any significant changes to the project, including the proposed method, consent process or recruitment procedures, will be reported to the College of Science Research Ethics Committee for consideration in advance of its implementation.

Signature of Principal Investigator	Ekhlass M. Taha Name of Principal Investigator	4-6-2022 Date
Signature of Student Investigator	Maryam Sabeeh Madhloom Name of Student Investigator	4-6-2022 Date

PART 10: DECLARATION BY COMMITTEE

Project Title:

Evaluation of the biochemical properties of Morus alba fruit extract and its healing effect on patients with eczema

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We certify that we read this proposal, and as scientific committee we examined the proposal contents and discussed the Information Sheet, as well as the Certificate of Constants, and we certify that study is performed in accordance with ethical standard as laid down in 1964 declaration of Helsinki statement and its later correction or comparable ethical standards, to the ministry of Iraqi health protocols.

Signature of Chairman of committee	Name of Chairman of committee	19-6-2022 Date
Signature of Member	Name of Member	19-6-2022 Date
Signature of Member	Name of Member	19-6-2022 Date
Signature of Member	Name of Member	19-6-2022 Date
Signature of Member	Name of Member	19-6-2022 Date