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Study of synergistic effect of Collagen and Licorice, Aloe Vera, Green Tea extracts in the treatment of burns in female Wistar rats

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ABSTRACT: In addition to the pain, burns can cost the injured person both financially and psychologically. In this experimental study, the alcoholic extract of aloe vera and green tea and licorice, which have been used in traditional medicine in Iran for thousands of years, along with collagen protein of fish skin individually, and then their double combination, triple combination, quaternary composition and Control was tested in 18 experimental boxes to treat skin lesions caused by Second degree burns on the skin of female rats. The results were then analyzed and then compared with a rat treated with a commercial ointment called silver sulfadiazineone1%, which is available in pharmacies and used for burns. The results showed that the combined use of herbs interferes with the treatment of burns, and others cause a synergistic effect and accelerate the treatment, and even faster than the commercial drug sulfadiazine silver.

Keywords: *Aloe vera, Collagen, Cytotoxicity, Green tea, Licorice, Side effects, Synergistic effect.*

INTRODUCTION

Surely all of you have experienced burn pain at least a few times in your life and you know how painful burn injuries can be. Early humans became acquainted with burns from the very beginning when they became acquainted with fire, and to this day, human beings are looking for ways to better treat burns.

Statistics show that one of the leading causes of death and disability in the world is burns and the resulting injuries. Burns are defined as injuries tissue caused by factors such as heat, chemicals, electricity, sunlight, or nuclear radiation [1]. Most burns are caused by contact with boiling water, building fires, liquids, water vapor, and flammable gases. Accelerating the healing process of burns and the quality of treatment has always been the focus of physicians. Because these wound are usually painful and heal later than usual. Therefore, proper treatment and care of these types of wounds to increase the speed of healing, as well as prevent their chronicity and infection has always been considered by physicians [2]. Burn injuries are more widespread in developing countries, leading to disability and long-term hospital-

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ization, and even death. Such injuries, regardless of the difficulty of treatment and their cost, can lead to abnormalities such as isolation or even depression. Statistics show that one of the most common causes of death in the world is burns, so that after road accidents and falls and collisions between people is known as the fourth group of accidents and unfortunately the burn rate in Iran is 8 times the global average [2]. This statistic shows us that more research needs to be done on burn treatment in our country. Many chemical drugs are used to treat burns today, and silver sulfadiazine is one of the most widely used. This drug with high antibacterial power is used against a wide range of microbial infections. But silver sulfadiazine, like many other chemical drugs, has harmful side effects that make doctors more cautious in prescribing such drugs. This drug causes keratinocytes to die due to cytotoxicity, which is one of the most important factors in wounds healing and delays wounds healing [3]. People who are allergic to sulfonamides should be more careful when using this cream. Chemical drugs used to treat burn injuries. In addition to allergies and cytotoxicity, they are usually expensive and unfortunately cause drug resistance in patients, and these problems have led scientists to seek alternative therapies to chemical drugs. Drugs of natural origin, such as herbs, were one of the best therapies that attracted the attention of scientists [4]. The nature of Iran, with its various natural climates such as mountainous deserts, deserts, forests, etc., has a great diversity in plant species, so that about 12,000 species of medicinal plants are known for the flora of Iran [5]. Green tee, which is found in abundance in Asia, contains compounds such as caffeine, catechins, flavonoids, vitamins A, B, C and fiber, glycoprotein's, polyphenols, lipids and carotenoids. Research shows that green tea consumption has many benefits such as anti-cancer, antioxidant, anti-aging; anti-inflammatory effects [6]. These main properties are due to polyphenolic compounds such as catechins in green tea leaves. Epicatechin, epigallocatechin, epigallocatechin gallate and epicatechin galactase are the main medicinal compounds in green tea with antioxidant properties (Fig. 1). Small amounts of these substances can increase collagen volume and rapid wound healing [7].

Studies have shown that epigallocatechin gallate in green tea can promote keratinocytes proliferation and differentiation [6]. In 2003, Chung *et al.* Showed that green tea extract (EGCG) causes the survival of epidermal keratinocytes and also has an anti-aging, anti-cancer effect on human skin [2]. Cell division in wound healing with green tea may increase due to the presence of compounds such as polyphenols, catechins, and EGCG. On the other hand, green tea is rich in vitamin C and has 18 types of amino acids, including proline and lysine, which are essential for the bonding of OH ions with the amino acids proline and lysine and their hydroxylation. In general, it causes cross-linking of amino acids in collagen and on the other hand increases angiogenesis, improves neutrophil function and macrophage migration [8].

Licorice root contains a variety of phytochemicals such as various sugars up to 18% flavonoids, sterols, gums, starches, amino acids, Essence oils and sa-

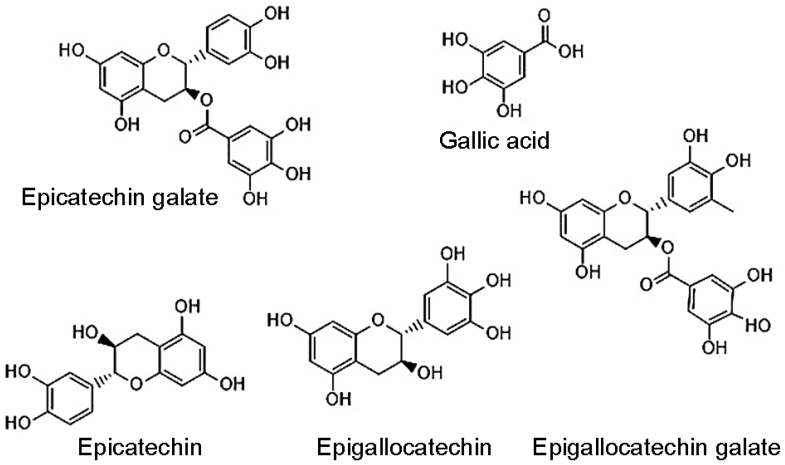

Fig. 1. A number of compounds in green tea.

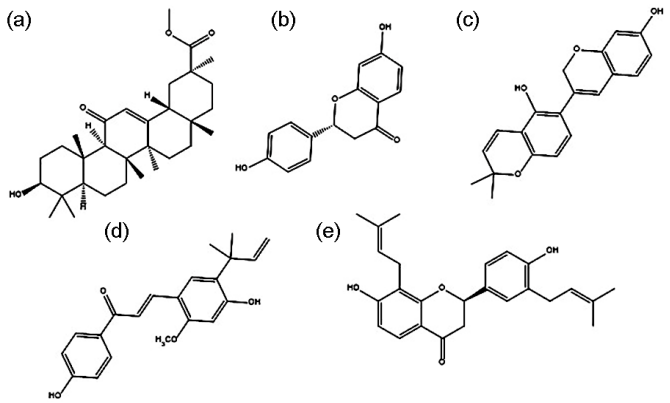

Fig. 2. Some compounds in licorice extract A: Glycyrrhizic acid/B: licorice Jenin/C: Galaburn/D: Lycochalcon a /E: Galabrol.

ponins. The main saponins are glycyrrhizic acid or glycyrrhizin. In addition to the above, licorice root contains 2-β-glucuronosyl acid-glucuronic acid, glycyrrhizic acid, asparagines, glucose, sucrose, volatile oils, resins, flavonoid compounds such as glycyrrhizic acid, Glycerin, glabrol, Lycochalcon a, licorice Jenin and Galaburn (Figure 2) major ingredient in licorice root, is fifty times sweeter than sugar. Glycyrrhizin also reduces pro-inflammatory cytokines and is known as an anti-inflammatory drug. Galabridine has antioxidant and anti-cancer properties. Lycochalcon A has antibacterial properties and Isolicorti jenin has anti-cancer and anti-inflammatory properties. Glabridin is an isoflavone of licorice root against gram-negative bacteria lipopolysaccharide compounds with anti-inflammatory and cardiovascular protective effects [9]. Galabron is a type of antihistamine that inhibits the enzyme cyclooxygenase inhibiting arachidonic acid and not converting it to histamine, serotonin and prostaglandins. By stopping the mentioned compounds, inflammation is reduced [10].

Researchers have identified 75 compounds in aloe vera that include 20 types of minerals, 20 types of amino acids, 12 types of vitamins and water [11]. Other important constituents of aloe include various sugars including: glucose, mannose and cellulose, enzymes including: oxidase, amylase and catalase, as well as vitamins such as: B1, B2, B6, C, E and folic acid and minerals and alumin Famudin, Anthrax Isobarbaline and its gum content is about 12% [12].

Aloe vera gel has a significant effect on accelerating the process of regeneration and reperfusion of burn tissue. These effects are thought to occur due to several mechanisms, including increased collagen synthesis and epithelialization by stimulation of fibroblasts, anti-inflammatory and antimicrobial effects. Aloe vera gel has been effective against a wide range of gram-positive and gram-negative bacteria. In addition to bacteria, the compounds in aloe vera are also effective on fungi and can be effective in dressing wounds against bacterial and fungal infections [2]. Katayoun Bagheri Mayab *et al.* (1397) studied the treatment of burns with collagen dietary supplement and the results of this study showed the consumption of high-calorie protein supplement based on hydrolyzed collagen (gelatin) for 4 weeks in patients with a burn rate of 20-30 % is effective in wound healing and increasing pre-albumin[13]. In this study, we also used fish skin collagen that we had obtained from a pharmacy to treat burns.

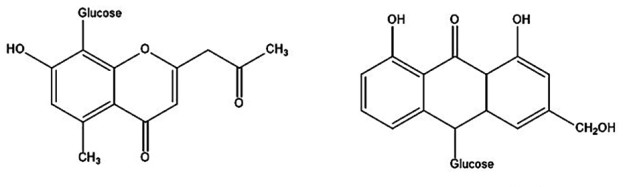

Fig. 3. Some compounds in Aloe vera extrac.

EXPERIMENTAL

Materials and Methods

Ethanol alcohol 96% Simin Tak Iran Company, Vaseline made in Iran is provided by Saj Company, Iranian green tea from Iran Gargood Company in Gilan, Woody roots of licorice plant from Lorestan region in

Iran, Natural collagen prepared from fish skin from the French company Weishardt, Aloe vera leaves grown in Pishva and Varamin regions in Iran, Ketamine anesthetic drug (Ketamine 10%) made in Beremer pharma Company in Germany, Xylazine anesthetic (Xylazine 2%) made in Alfasan Company in Netherlands, Silver sulfadiazine-Nago 1% ointment made by Nazho Pharmaceutical Company in Iran, 50 female Wistar rats prepared by Pasteur Institute of Iran, Plate food prepared in the Parvar Company, Russian wood straw as a substrate (Bought from a carpenter in Varamin).

RESEARCH METHOD

Licorice extract

First, the woody roots of the licorice plant were cut into small pieces with scissors, and then they were poured into a mill and after 20 minutes, they became powder. Then, 200 grams of ground material was poured into an Erlenmeyer flask and 1 liter of 70% ethanol was added to it, and the resulting mixture was stirred vigorously. The mixture was kept at room temperature for 2 days and after purification with filter paper, the pulp was hardened and an alcoholic solution was obtained. The solution containing alcohol was extracted for 1 hour in a vacuum distillation apparatus at 50 ° C, and then the extract was dried in an oven for 2 days at 50 ° C. The dried extract was ground in a mortar and ground into a powder [10].

Aloe Vera extract

600g of aloe vera leaf gel was separated by a grater and kept in Erlenmeyer with 1 liter of 70% ethanol af-

Table 1. Treatments and amount of drug used in each test.

Row	Abbreviation of treatment	Type of treatment	Ingredients of treatment	Description
1	O	Control	No treatment	Does not have any treatment or matrix
2	VG	Control	Vaseline + glycerin	Control - no drug only matrix (Vaseline + glycerin)
3	C	Control	Collagen	Collagen=4 g
4	A	Control	Aloe Vera	Aloe Vera=300 mg
5	L	Control	Licorice	Licorice=700 mg
6	G	Control	Green tea	Green tea=400 mg
7	P	Control	Silver sulfadiazine	Silver sulfadiazine 1%
8	CL	Dual treatment	Collagen + Licorice	Collagen=4 g / Licorice=700 mg
9	CA	Dual treatment	Collagen + Aloe Vera	Collagen=4 g / Aloe Vera=300 mg
10	CG	Dual treatment	Collagen + Green tea	Collagen=4 g / Green tea=400 mg
11	LG	Dual treatment	Licorice + Green tea	Licorice=700 mg / Green tea=400 mg
12	LA	Dual treatment	Licorice + Aloe Vera	Licorice=700 mg / Aloe Vera =300 mg
13	GA	Dual treatment	Green tea + Aloe Vera	Green tea=400 mg / Aloe Vera= 300 mg
14	ACL	Triple treatment	Aloe Vera + Collagen + Licorice	Aloe Vera=300 mg/ Collagen=4 g / Licorice=700 mg
15	GCA	Triple treatment	Green tea + Collagen + Aloe Vera	Green tea =400 mg / Collagen=4g / Aloe Vera=300 mg
16	LAG	Triple treatment	Licorice + Aloe Vera + Green tea	Licorice= 700 mg / Aloe Vera=300 mg/ Green tea=400 mg
17	LGC	Triple treatment	Licorice + Collagen	Licorice=700 mg / Green tea=400 mg / Collagen=4 g
18	GLAC	Four treatments	Green tea + Licorice + Aloe Vera + Collagen	Green tea =400 mg / Licorice=700 mg / Aloe Vera=300 mg/ Collagen=4 g

ter stirring for one minute (manual shaking) for 2 days at room temperature, then filtered through a 150 micron sieve. The resulting liquid was vacuum distilled at 50 ° C for 1 hour and then dried in an oven at 50 ° C for 72 hours. The extract was ground and powdered in a mortar [11].

Green tea extract

200 g of green tea leaves in 1 liter of 70% ethanol was kept at room temperature for 2 days and then the pulp was removed by filter paper and the alcoholic solution obtained in the vacuum distillation apparatus was distilled at 50 ° C for 1 hour. And the solution was concentrated. The resulting solution was dried in an oven at 50 ° C for 48 hours and the extract was ground and powdered in a mortar [12].

Preparation of treatments

First, according to the description, plant extracts were prepared and then poured into small containers with a volume of 200 mL and the rest of the volume was filled with Vaseline and glycerin. It was thoroughly mixed. (1.5 liters of Vaseline was mixed with 150 ml of glycerin to help the solubility of the ingredients in Vaseline). The Table below shows the amount, type and abbreviation of each drug. In the continuation of this research, we will use the abbreviations used in Table 1 to introduce each box.

VG= Vaseline + glycerin, A= Aloe Vera, L= Licorice, G= Green tea [8], C= Collagen, O= Control[i]

[i] (Some of the numbers in Table 1 were considered based on previous research, such as the amount of green tea extract from Yaghmaei *et al.* [8]. And due to the novelty of the subject and the lack of similar research in this field, we had to determine the rest of the numbers in Table 1 based on the toxicity of the extracts and inspiration from the research of others. Obviously, in order to achieve the optimal percentage of test compounds, extensive research had to be done, which was beyond the scope of this study).

The treatments were poured into 100 ml sealed containers and the rest of the container was filled with Vaseline + glycerin then mixed well. After making the treatments, rats were anesthetized by peritoneal injection with 4 Units of insulin ketamine and 6 Units of insulin xylazine. After shaving the hair in the waist area and disinfecting the waist area with beta dine by a piece of round steel with a diameter of 3 cm and a temperature of 120 °C for 5 seconds and were burned and a second degree burn was induced. Rats were divided into boxes 2 and 3 immediately after the burn, and one rat was considered for commercial drug treatment. And each box received its treatment for 20 days daily at a specific time between 10-12 in the morning. During all this time, the rats had extra food and their litter was changed every two days.

Twenty days later, the rats were eradicated by injection of 3 mL ether. Then, their burn injuries were measured by calipers and their recovery percentage was calculated according to the following formula.

$$\text{Percentage of wound surface per day x} = \frac{\text{Wound area on day x} \times 100}{\text{Wound area on day zero}}$$

Percentage of healing per day x=100-Percentage of wounds per day x

RESULTS AND DISCUSION

After calculating the test results according to the formula mentioned earlier, each individual team improved the calculation and the results were as follows. The results of the present study showed that the alcoholic extracts of licorice, aloe vera, green tea and collagen from fish skin can be useful in treating burns and speed up the healing process. The results indicate that the use of alcoholic extract of green tea, despite the phenolic compounds in alcoholic extract, can increase the speed of healing of burn wounds by increasing the construction of blood vessels in the skin and its anti-inflammatory properties.

In this study, it was observed that the combined use of two treatments reduced the therapeutic effect and probably caused harmful compounds in the treatment of burns. On the other hand, in most cases, each of the drugs used alone at a particular stage is more effective in treatment. For example, aloe vera at the beginning of treatment caused the wound to dry well and the environment became unbearable for pathogens and Collagen treatment was not very interesting at the beginning of the burn treatment, but after ten days it miraculously healed the wound And the treatment

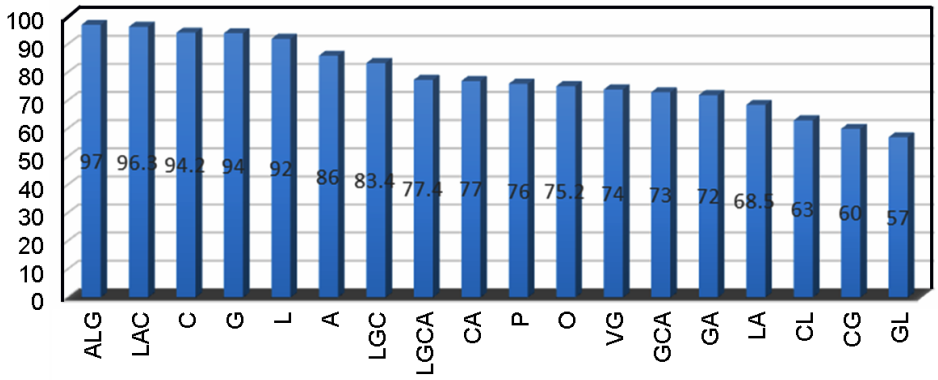

Fig. 1. Graph of recovery percentage on the twentieth day of treatment.

with green tea was like collagen, but at the beginning of the treatment, it dried and disinfected the wound, and after the scab had fallen, it improved the speed of healing. The results of this study, which are shown in the form of diagrams, showed that rats that used commercial drugs had a longer treatment period than rats that used ACL, ALG, C, GL, A, LGC therapies, which could be due to the cytotoxicity of such drugs (Appendix 2 ,3). Be. Chemical drugs containing silver and sulfadiazine act on a wide range of bacteria and infectious agents, but have destructive side effects that kill young, newly formed cells at the site of injury and delay keratinocytes and skin repair cells by delaying skin repair.

In this study, statistics showed that in comparison with the treatments used individually, all of them had a positive effect in the treatment of burn injuries, but among them, green tea plant extract was more effective and faster than other plant extracts. Of course, the collagen function was excellent in the third week and performed better in repair than all herbal treatments. It seems that a mixture of aloe vera and licorice can improve the therapeutic effects of collagen and green tea and have a positive synergistic effect. However, in GLAC treatment, it was observed that the combination of green tea with collagen can greatly reduce the therapeutic effect of this treatment and, like other treatments that had collagen GC and alcoholic extract of green tea, had a relatively low speed of treatment. It seems that GC treatments aggravate inflammation in injury. In LGC treatment, licorice in this treatment has reduced the inflammatory effects of GC treatment. In Appendices 1, 2 and 3, you can see the treatment on the twentieth day.

CONCLUSIONS

Concomitant use of alcoholic green tea extract and fish skin collagen (CG) can be dangerous when treating burn injuries because, as we have seen, it can have a detrimental effect and delay healing. In some cases, such as ALG and ACL, the treatment process responded even better than individual treatments, and we saw about 1.8 to 3% positive synergistic effects in treatment with these treatments. In this study, we sought a synergistic effect between the treatments of this study and showed that the use of these treatments as a drug can work even better than commercial drugs on the market, and this study showed that medicinal plants in Iran have the potential to treat burn injuries. They have the right speed and quality and their right combination can show extraordinary therapeutic effects.

ACKNOWLEDGEMENT

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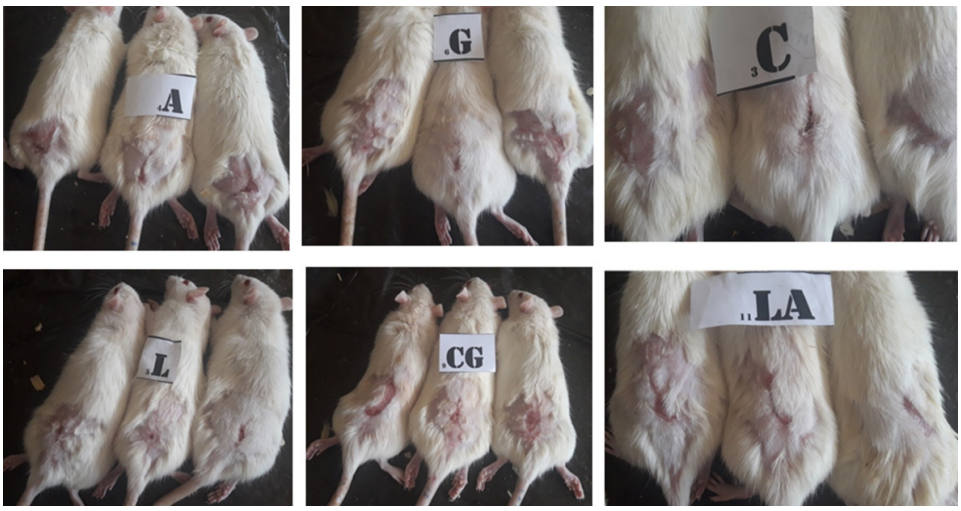
This research has the code of ethics: IR.IAU.VARAMIN.REC.1399.036 dated 2020/10/3 approved in the work of the research ethics committee of the Islamic Azad University, Pishva-Varamin branch.

Appendix 1

Appendix 2



Appendix 3

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