

Antiaging Effects of a Poly Herbal Extract in Comparison with Vitamin E on Aging Induced Mice

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ABSTRACT

Background: Herbal medicine has been used from ancient times to defy aging as Cleopatra used several herbs as a drink or topically and pomegranate was a main herb used in her system, it was named the fruit of immortality. Combining three herbs into one preparation is recommended to increase medicinal benefits while reducing toxicity and to obtain a synergistic effect. In this study we have found that a polyherbal (*Apium graveolens*, *Carthamus tinctorius*, *Punica granatum*) extract from Iraqi origin have gave a stronger effect than vitamin E.

Aim: The aim of this study was to evaluate the effect of the poly herbal extract *in vitro* as an antiaging and antioxidant agent in comparison with vitamin E, which is considered as one of the most potent anti aging agents.

Materials and methods: forty mice were orally treated and divided into 4 groups (all were induced with D- Galactose for aging accept the positive control): the first group was positive control, the second was negative control while the third group was treated with vitamin E, and the last group with the polyherbal extract. Later a number of biochemical tests were conducted to evaluate its effectiveness including; test of catalase, GSH-PX, GSH and oxidative stress parameters

Results: Our research showed that treated groups with the herbal extract showed great results compared the group treated with vitamin E. Lower levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) were shown to be associated with reduced levels of MMP-1 upregulation, collagen loss, and increased levels of Nrf2 nuclear accumulation. The polyherbal extract we found, upregulated Nrf2 and reduced ROS production and oxidative damage by D- Galactose.

Conclusion: According to the results of our research, the polyherbal extract may be a promising candidate for the prevention of aging signs induced by the intake of D- Galactose. The antioxidant action of vitamin E (VE) makes it an essential nutrient and an important antioxidant to defy aging. Deficit (VED) of VE leads to central nervous system, reproductive and immunological disorders leading to aging. However, this study showed that the polyherbal extract we used gave much better results than vitamin E in preventing aging signs.

Keywords: Biochemical analysis, *Apium graveolens*, *Carthamus tinctorius*, *Punica granatum*, Antiaging

INTRODUCTION

There is evidence that glycation products accumulate in slow-renewing tissues, such as the skin, and that this is a key factor in the aging process of the skin. It's common practice in the clinic to employ adipose-derived cells to treat ischemia illnesses and speed up the healing process after wounds. The mechanism behind the effectiveness of adipose-derived stem cells (ASCs) n anti-aging treatment is yet unclear, which is interesting. The study's goal was to see if ASCs had an anti-aging effects in an animal model of aging caused by D-galactose, and if so, what the underlying mechanism was (1). on the other hand, are known to have anti-aging effects on the skin. Antioxidants are significant nutraceuticals with health benefits and antiaging cosmetics ingredients because of this. Current aging models include D-gal-induced aging, senescence-accelerated mouse (SAMP) model, and natural aging model in rats, mice, and other animals (2). In comparison to other animal aging models, the D-gal animal model's modeling technique is straightforward, low-cost, and reliable. The D-gal animal model is now a well-known and commonly utilized one in the research of anti-aging medications all over the world. Animals are continually injected with D-gal for a period of time, resulting in a significant rise in intracellular galactose concentration *in vivo*. Because oxidative stress is a factor in both intrinsic and extrinsic causes of skin aging, treating skin aging and its complications with anti-oxidants may be a beneficial strategy. Melanocytes, the specialized cells that secrete melanin to provide the skin color, are the sites of malignant melanoma, a kind of cancer. Every year, melanoma affects 132,000 people all around the world (3). Malignant melanoma is currently treated with a combination of surgical resection, radiation treatment, immunotherapy, chemotherapy, and targeted therapy. *Aegle marmelos* (often referred to as bael) and *Musa paradisiaca* (sometimes referred to as banana) both contain polyphenolic chemicals that may be useful in the treatment of age-related disorders as well as skin cancer. These compounds are particularly useful in preventing and treating skin cancer. A major food crop in the globe, *Musa paradisiaca* has a wide range of

health benefits, including being leishmanicidal, antioxidant, anti-ulcerogenic, anti-hyperglycemic, and anti-coccidial (4). However, no research has been done on the anti-aging and anti-carcinogenic qualities of *Aegle marmelos*, *Nyctanthes arbor-tristis*, or *Musa paradisiaca*. Apart from that, till date, no formulation has been examined that incorporates the aforementioned properties of plant extracts. These fruits, as well as the leaves of *Nyctanthes arbor-tristis*, *Musa paradisiaca*'s terminal meristem, and the unripe pulp of *Aegle marmelos*, were studied for their anti-oxidative qualities. (5). It includes everything from moderate cognitive impairment all the way up to the neurodegenerative disorders such as Alzheimer's, stroke, heart disease, and Parkinson's. Memory declines with age, but not semantic memory or general knowledge like vocabulary definitions, which rises until late adulthood and then stays constant. As people become older, their intelligence deteriorates, albeit the rate varies depending on the type and may even remain constant for the most of the lifespan before rapidly declining towards the end. Since the free radicals created by mitochondrial activity destroy cellular components, this is referred to as the "mitochondrial hypothesis of ageing."DNA oxidation and caloric restriction have been linked in experiments, with caloric restriction reducing 8-OH-dG DNA damage in the organs of aged rats and mice (but not in young ones) (6). The stalk of celery, which is long and woody, tapers to leaves at the end. The stalks, leaves, or hypocotyl of this plant can be eaten raw or cooked, depending on the region and cultivar. Aside from culinary use, celery seed is also used as a spice and in herbal medicine (7). To treat different medical disorders, such as dysmenorrhea, amenorrhea, postpartum abdominal discomfort, and mass, the herb *Carthamus tinctorius* L., also known as Kafesheh (Persian) or Safflower (English), is widely used in Traditional Chinese Medicine (8). *Punica granatum* There are two subfamilies of the pomegranate (*Punica granatum*), both of which yield fruit and grow between 5 and 10 meters (16 and 33 feet) tall in the Lythraceae family, subfamily Punicoideae. Pomegranates have been used in natural and holistic medicine to cure a variety of ailments, including sore throats, coughs, urinary infections, digestive problems, skin

problems, arthritis, and tapeworm infestations. Those who are sensitive to pomegranate extract may want to avoid it. Itching, swelling, a runny nose, and trouble breathing are all signs of sensitivity. Taking significant amounts of pomegranate root, stem, or peel may be harmful. Poisons are found in the root, stem, and peel (9).

MATERIALS AND METHODS

Six-week-old mice received daily subcutaneous injections of D-gal for eight weeks. A placebo was administered to the mice in the control group, which received no treatment. Histopathology as used to look at the molecular markers linked with hepatic aging, such as SOD and malondialdehyde (MDA).

A position paper on ethical principles: The college of medicine-University of Nahrain's Laboratory Animal Administration Committee approved animal experimental techniques in accordance with the university's Guidelines for Animal Experimentation. The animals were spared as much pain and suffering as possible by all means.

A study of the numbers: Quantitative and morphometric analyses produced mean values and measurement standard errors (SEM). We utilized SPSS 13.0 for statistical analysis (SPSS Inc., Chicago, IL). The data were compared using the ANOVA test, with the LSD test being employed as a post-hoc analysis as required. There must be a P-value of less than or equal to 0.05 for anything to be considered statistically significant

Experimental animals: 40 Wistar mice weighing between 20-25g will be used and will be housed in standard environmental conditions (12h / 12h light and dark) at 25 ± 3° C. They will be fed with standard rat pellet and water.

Induction of aging: Induction of aging in mouse model by subcutaneous injection 0.3 mL of D-galactose (500 mg/kg), once daily for 8 week and keep observation for the development of signs of aging in the Negative and positive control and treatment groups.

Plant extraction: The three plants (Apium graveolens, Carthamus tinctorious and Punica granatum) were collected from the local market in Baghdad, then washed by tap water and air-dried in the shade, and powdered then extracted in a Soxhlet with water. Then the final product will be filtered and excess water was evaporation in a rotary evaporator and the solid mass was stored until used. Study groups: 40 mice divided into 4 equal groups:

1. Group 1: Healthy control: Normal saline was given orally once daily for 6 weeks. Aging was induced in the rest of the mice and will be subdivided into the following:
2. Group A: Aging induced group (negative control): Normal saline was given orally once daily for 6 weeks.
3. Group B: Treatment with (100 mg/kg vitamin E) via intragastric administration once daily for 6 weeks from the third week (Positive control).
4. Group C: Mice were treated orally with the poly herbal formulation for 4 weeks.

Biochemical analysis: Analysis of enzymes and chemical compounds in blood and tissues of euthanized mice for different treatments.

Oxidative stress Parameters: The enzymes and compounds to be detected are glutathione (GSH), glutathione peroxidase (GSH-Px) of tissues and serum, catalase (CAT), malondialdehyde (MDA). Aging parameters: Skin collagen, elastin, hyaluronic acid and moisture content.

RESULTS AND DISCUSSION

Animal food consumption and body weight did not differ significantly between the two tests. No mice died in the course of the experiment before they were euthanized. A listlessness, lag in reaction, poor mobility, and withered and lackluster fur were all seen in mice in the D-gal model group. These organ indices were considerably lower in model group members than the control group members. There was a considerable increase in the MDA content in the model group's brain compared to the control group, showing

that the aging animal model had been effectively established while treatment group with the polyherbal extract showed lower MDA contents than the model group. group had much lower levels of MDA in their livers, kidneys, and serum than did the control group. Furthermore, intragastric treatment lowered the MDA skin levels in aged mice.

These effects are comparable to AG, which is a potent inhibitor of the AGE-forming process. The production of angiogenic factors like vascular endothelial growth factor by ASCs in vivo further suggests that the skin is acting as a trophic factor in this regard as well (10). Aging is a complex and unavoidable natural process when seen from a biological standpoint (11).

Table 1: Serum levels of Uric acid, urea, creatinine and ATP.

Groups	Uric acid	Urea	Creatinine	ALP
C-	3.99±0.1 1e	34.78±3. 03b	0.62±0.03 e	81.03±4.5 5e
C+	4.92±0.1 8a	41.04±2. 99a	0.92±0.04 a	130.25±4. 97a
D-gal.+Vit.E 100mg	4.76±0.2 0b	39.12±3. 03a	0.82±0.02 b	130.25±4. 97a
D-gal.+combination 200mg	3.95±0.1 4e	34.87±3. 30b	0.63±0.02 e	86.71±9.7 2de
LSD*	0.15	2.81	0.02	6.74

Means with a different letter in the same column are significantly different (P<0.05)

* Least Significant Difference (LSD)

People throughout the world are becoming more self-conscious as their quality of life rises. People are becoming increasingly concerned with delaying the onset of skin and bodily aging. As people live longer lives, their tissues and organs begin to atrophy and lose functionality. The weight of the brain and kidneys have changed significantly. As a result, changes in the organ index are an essential sign of aging in an organism. According to our research, D-gal can cause premature aging in the model animals by reducing organ indices such as those for the brain, liver, and kidney. The polyherbal extract was able to enhance all organ indices when compared to the model group. It showed that the polyherbal extract could preserve those organs' weight from declining as they aged due to D-gal and had a clear antiaging impact on the brain, liver, and kidney. As a result of living in an oxygenated environment, cells have had to evolve efficient detection and detoxification methods for ROS metabolites. When ROS are created, they can damage a variety of cellular components and set off a cascade of signaling pathways, but they can also have critical downstream consequences.

Table 2: Serum levels of ALT, AST, TG, and Cholesterol

Groups	ALT	AST	TG	Cholesterol
C-	62.00±6.4 5 ^c	116.97±11 .49 ^{cd}	76.86±4.03 ^a	74.75±3.49 ^f
C+	122.55±7. 47 ^a	147.21±13 .27 ^a	134.37±6.14 ^a	122.11±5.1 9 ^a
D-gal.+Vit.E 100mg	120.11±10 .36 ^{ab}	131.10±11 .72 ^b	126.70±5.42 ^b	115.11±5.9 6 ^b
D-gal.+ combinatio n 200mg	73.52±8.7 8 ^d	116.33±13 .50 ^d	89.82±6.10 ^f	85.24±5.02 ^e
LSD*	7.93	11.26	4.79	4.39

Means with a different letter in the same column are significantly different (P<0.05)

* Least Significant Difference (LSD)

When these things happen, they can dramatically alter several physiological processes and metabolic pathways that are linked to the aging process, whether it's internal or external (such the development of age-related diseases or skin aging). Excess ROS, for example, can lead to lipid peroxidation in the body. MDA is the end product of the oxidation process. MDA has cytotoxicity and can direct protein and nucleic acid cross-linking. As a result,

the amount of MDA in the blood may represent both the body's degree of lipid peroxidation and the amount of cell damage caused by reactive oxygen species (ROS). Most of the negative effects of ROS can be eliminated by a comprehensive antioxidant defense system that includes the key enzyme scavengers CAT, and GSH-Px (12).

GSH-Px has the ability to convert hazardous peroxide into a harmless hydroxyl molecule, protecting the cell membrane's structure and function from peroxide damage. CAT can breakdown H₂O₂ into molecular oxygen and water in vivo, protecting cells from the oxidative damage caused by H₂O₂, and then remove the hydrogen peroxide. CAT may be present in all known animal species, with concentrations being highest in the liver. Different nonenzymatic and low-molecular-mass compounds, such as pyruvate and ascorbate can scavenge ROS, as well as polyphenols and flavonoids. The molecules glutamic acid, cysteine, and glycine are used to create glutathione (GSH), one of these molecules. Every cell in the body contains GSH, which is a powerful antioxidant and detoxifier. GSH has the ability to not only neutralize free radicals in living cells, but it may also boost an organism's overall immunity. A person's health is closely linked to their body's antioxidant defense system's ability. Free radicals produced in vivo by D-gal metabolism, as previously mentioned, are thought to contribute to aging. In other words, a compound that is highly effective at scavenging free radicals also possesses antiaging properties. Furthermore, no mice perished as a result of D-model-making gal's efforts. It was therefore possible to create realistic aging models. We found that orally administered polyherbal extract improved the antioxidant defense system of aged mice by increasing the activities of CAT, and GSH-Px in tissues and serum while decreasing MDA levels. D-gal causes morphologic damage to the brain, liver, and kidneys, which oral administration of the polyherbal extract can help reverse. It can also be named as "beauty drink" or "beauty from inside" or even "oral cosmetics" in the new idea. Oral poly herbal extract treatment may also enhance the skin condition of elderly mice by increasing Hyp content, for example (2). In vitro and in vivo, it was well-known that VE had more antioxidant activity; nevertheless, our investigations showed that polyherbal extract had a better level of antioxidant activity.

Damage from ROS may lead to an increase in melanocyte overproduction and elastin and collagen consumption in the skin. These injuries also reduce the skin's capacity to regenerate. Cumulative effects include skin wrinkling and fragile look as well as brown pigmentation that is mottled and noticeable dark blotches. Antioxidant supplementation, whether oral or topical, is intended to boost the body's own supply of antioxidants, not to eliminate free radical generation totally. Instead, it serves to boost the body's natural defenses in order to avoid further injury. The

Table 3: Serum levels of HDL, LDL, VLDL, MDA

Groups	HDL	LDL	vLDL	MDA
C-	32.33±3.8 6 ^a	27.04±4.4 9 ^f	15.37±0.8 0 ^g	6.82±0.58 ^e
C+	16.23±1.9 4 ^d	78.99±5.5 9 ^a	26.87±1.2 3 ^a	11.28±1.29 ^a
D-gal.+Vit.E 100mg	16.56±2.2 5 ^d	73.20±6.9 7 ^b	25.34±1.0 8 ^b	10.82±1.22 ^{ab}
D-gal.+ combination 200mg	32.35±3.7 0 ^a	34.92±5.2 4 ^e	17.96±1.2 2 ^f	6.08±1.74 ^d
LSD	3.14	5.56	0.95	1.99

Means with a different letter in the same column are significantly different (P<0.05)

dermis contains significant amounts of collagen fibers (most of which are composed of collagen). Collagen fiber content decreases considerably as we get older, resulting in sagging, rigid skin. As a result, changes in collagen content can influence how quickly skin ages. Because of the high amount of Intradermal Hyp content, it may be able to reveal changes in collagen fiber number

and, thus, the dermal aging process. Hydroxyproline makes up 13.4% of the total amino acid content in collagen, a negligible amount in elastin, and is absent from all other proteins. The hyaluronic acid (HA) content of the skin is used as a sign of how old the person is. As a result, many individuals are under the impression that replenishing collagen is the key to keeping skin young, but in fact, elastin is just as vital. Elastin plays an important role in skin's elasticity and suppleness. People cease producing elastin around the age of 25, thus the skin loses its elasticity and begins to droop. Women are known to lose elastin at a higher rate than males. As a result, a rising number of women are preoccupied with antiaging skin care. Skin moisture content also plays a critical regulatory role in the skin's physiological operations. Skin tightness, peeling, and itching are the most common signs of dryness on the face and body. As a result, a rising number of women are interested in moisturizing cosmetics. Fortunately, our delaying-skin-aging experiment found that the polyherbal extract was helpful in improving the state of mouse skin (13). Collagen, elastin, and moisture content were all maintained while MDA levels were reduced in the aged mouse skin. The morphological alterations in mouse skin have clearly demonstrated the poly herbal extract anti-aging action. In all trials, the polyherbal extract was able to reduce MDA content efficiently, indicating that it exhibited strong antilipid peroxidation efficacy. According to our findings, polyherbal extract slows down the aging process by increasing the body's antioxidant capacity. To make things even more interesting, it had a greater impact than the well-known VE. As a result of these findings, it appears that our work holds considerable promise as a source of antiaging medication and cosmetics.

Table 4: Serum levels of catalase, GSH- PX and GSH

Groups	Catalase	GSH-PX	GSH
C-	349.60±13.79a	118.73±5.45a	4.42±0.65a
C+	288.56±9.80d	91.53±3.73d	2.95±0.32e
D-gal.+Vit.E 100mg	292.92±15.84cd	93.20±5.09d	3.16±0.32de
D-gal.+ combination 200mg	324.97±10.04b	110.39±5.57b	4.10±0.31ab
LSD	12.08	4.84	0.39

Means with a different letter in the same column are significantly different (P<0.05)

* Least Significant Difference (LSD)

CONCLUSION

As a result, phytochemicals found in the leaves of *Nyctanthes arbor-tristis* and PHF1 have anti-oxidant activity that might be employed to help prevent skin aging and restore skin elasticity in older adults. The anti-cancer properties of PHF1 make it an attractive candidate for use in treating malignant melanoma. Purification and characterization of phytochemicals found in plant extracts, as well as a detailed investigation of PHF1's anti-cancer efficacy in cell culture models, require further research. These findings suggest that ASCs may play a role in skin regeneration as we become older. There's also evidence to suggest that ASCs have antioxidative, glycation-suppressive, and trophic benefits in an aging mouse model, according to the study's findings.

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