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Review Article

18β-Glycyrrhetinic acid: its core biological properties and dermatological applications

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Abstract

Recently, attention has been focused on identifying natural herbal compounds with high biological activity, especially antioxidative, anti-inflammatory and antimicrobial properties, for preventing and controlling various skin conditions, including inflammation-related diseases such as atopic dermatitis and UV-induced skin photoaging. One key active plant ingredient is 18β-glycyrrhetinic acid (GA), the main metabolite of glycyrrhizin (GL), obtained from licorice root. The review examines the valuable biological properties of GA, particularly those playing key roles in the treatment of various dermatological disorders in humans. The review highlights the key anti-inflammatory, antioxidant and antimicrobial properties of GA and its toxicity towards normal cells lines. It also examines the physicochemical properties of GA and presents methods of increasing its penetration through the stratum corneum and bioaccumulation with the use of modern delivery systems such as liposomes and nanoemulsions.

Résumé

Récemment, l'attention s'est concentrée sur l'identification de composés naturels à base de plantes ayant une activité biologique élevée, en particulier des propriétés antioxydantes, anti-inflammatoires et antimicrobiennes, pour prévenir et contrôler diverses affections cutanées, y compris les maladies liées à l'inflammation telles que la dermatite atopique et le photovieillissement induit par les UV. Un ingrédient actif végétal clé est l'acide 18*β*-glycyrrhétinique (GA), le principal métabolite de la glycyrrhizine (GL), obtenu à partir de la racine de réglisse. La revue examine les propriétés biologiques précieuses de l'AG, en particulier celles qui jouent un rôle clé dans le traitement de divers troubles dermatologiques chez l'homme. La revue met en évidence les propriétés anti-inflammatoires, anti-oxydantes et antimicrobiennes essentielles de l'AG et sa toxicité vis-à-vis des lignées cellulaires normales. Il examine également les propriétés physicochimiques de l'AG et présente des méthodes pour augmenter sa pénétration dans la couche cornée et sa bioaccumulation grâce à l'utilisation de systèmes d'administration modernes tels que les liposomes et les nanoémulsions.

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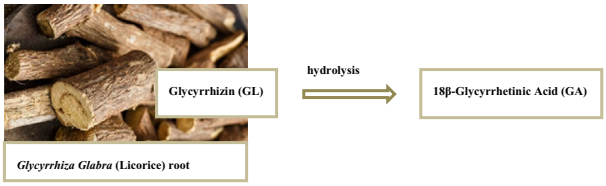
Introduction

The growing trend of "natural cosmetics" has resulted in increasing use of natural substances in the production of cosmetics. Of these naturally-derived substances, 18β-glycyrrhetinic acid (GA) is of special importance. It is characterized by a broad spectrum of antioxidative, anti-inflammatory and antimicrobial activities, among others, which makes it suited for many possible applications in dermatology and cosmetology. Additionally, allergic contact dermatitis is very rarely observed associated with the use of glycyrrhetinic acid [1].

18β-Glycyrrhetinic acid (GA), 3β-hydroxy-11-oxo-18β,20β-olean-12-en-29-oic acid, Enoxolone, INCI: Glycyrrhetinic acid Fig. 1) is the major active component of licorice root extract, which contains from 2% to 25% GA, mainly as glycosidic glycyrrhizic acid (GL) (Fig1) [2].

Licorice (*Glycyrrhiza glabra* L.), also known as Yashtimadhu, gancao, Grandfather Herb, and Sweetwood or yasti-madhu, is one of the most common herbs in Chinese herbal medicine, with a very long history of use [3,4]. The herb is widespread, growing in southern Russia, central Asia, the Mediterranean region, northern China and America [5]. Other ingredients derived from *Glycyrrhiza* species include other saponins, flavonoids such as flavanones, chalcones, flavons, isoflavons and isolavans, as well as coumarins and other phenolics [6].



Glycyrrhizic acid (GL or glycyrrhizin), a triterpenoid saponin glycoside, is the major water-soluble constituent of licorice root, while 18β-glycyrrhetinic acid (GA or glycyrrhetic acid) is the key metabolite of glycyrrhizic acid (Fig. 1). During metabolism in the plant by glucuronidase, or by intestinal bacteria after oral ingestion, glycyrrhizin is hydrolyzed into two pentacyclic triterpenoids, which are stereoisomers: 18α- and 18β-glycyrrhetinic acids [7].

From a biological perspective, the pentacyclic triterpenoids (PTs), bearing a basic molecular structure with five rings, have attracted

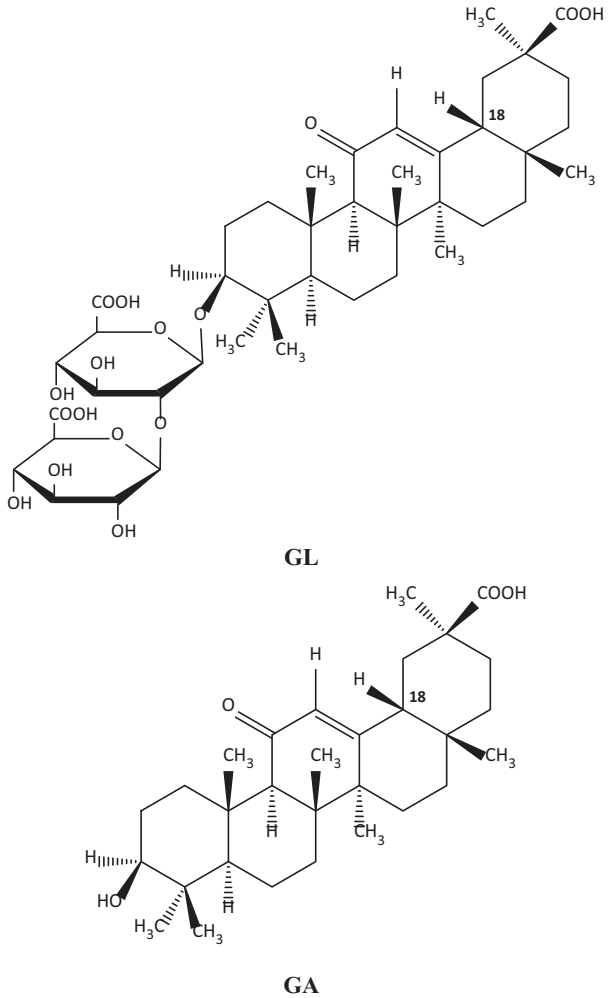


Figure 1 Chemical structures of glycyrrhizic acid (GL or glycyrrhizin) and 18β-glycyrrhetinic acid (GA or glycyrrhetic acid).

much attention due to their pharmacological properties. So, 18β-glycyrrhetinic acid (GA) and its derivatives exhibit a remarkable broad spectrum of biological and pharmacological activities, including antitumor [8,9], anti-inflammatory [10,11], antioxidant [12], antiviral [13], antimicrobial [14], antiulcer [15], antidiabetic, hepatoprotective [7,16], cardioprotective and neuroprotective effects [3].

The aim of the review is to present the anti-inflammatory, antioxidant and antimicrobial activities of GA which are of particular importance for its applications in dermatology and cosmetology. It also investigates the physicochemical properties of GA and presents strategies using modern delivery systems such as liposomes and nanoemulsions intended to improve its penetration through the stratum corneum.

The physicochemical properties of GA, and strategies to enhance its transdermal delivery

GA has some undesirable physicochemical properties, including inadequate lipophilicity, poor bioavailability and low water solubility, which drastically decrease its percutaneous absorption profile [17,18]. Fortunately, there are many permeation enhancement strategies for improving the bioavailability of this interesting compound.

It is widely acknowledged that skin penetration is dependent on the logarithm of partition coefficient (log *P*), which is an indicator of lipophilicity of a compound, and its molecular weight. This is caused mainly by the lipophilic properties of the outer, dead layer of the skin: the stratum corneum. Compounds with a log *P* value of approximately 1 to 4 and molecular weights less than 500 Da can easy penetrate through the skin [19], and drugs used for topical treatment are designed accordingly. Improving the bioavailability of the compounds leads to greater efficacy. Although the molar mass of GA is less than 500 Da, more precisely 470.7 Da, its log *P* value is quite high (6.574), therefore a strategy to increase GA permeability is needed [17,20].

To overcome the barrier of the stratum corneum and enhance the transdermal delivery of active ingredients, various methods have been developed. One of which involves the application of modified derivatives; however, the use of such structurally-modified compounds also carries the risk of poor skin tolerance [21]. Another method is based on trapping compounds in lipophilic carriers such as liposomes; the physical properties of liposomes, such as their size, charge, lamellarity and elasticity, play a crucial role in the penetration process [22].

Li *et al.* confirm that elastic liposomes (EL), highly-versatile deformable liposomal systems, are very effective and stable carriers for the transdermal delivery of GA for the treatment of chronic contact dermatitis. EL combined with GA was found to offer fivefold greater efficiency than conventional liposomes and saturated solutions, while EL combined with ethanol displayed 23-fold greater efficiency. The EL also easily passed through 50 nm pores [18,23].

In addition, an elastic liposomal system containing lysine has been developed to enhance the solubility and skin permeation of the poorly water-soluble GA. The study examined the efficacy of a gel containing flexible GA liposomes for the treatment of percutaneous dermatitis. The incorporation of lysine resulted in a 11.6-fold increase in the liposomal load of the drug compared to the administration of the drug without lysine. Percutaneous absorption from liposomal gels increased proportionally as the dose increased from 0.3 to 0.9%, indicating linear pharmacokinetics in a dose-dependent manner. GA 0.9% liposomal gel demonstrated a stronger anti-inflammatory effect among mouse models with contact dermatitis induced by DNFB (2,4-Dinitrofluorobenzene); TAEN cream was used as a reference positive control. Additionally, the amount of GA in the liposomal gel remained constant during storage for six months [24].

To improve the performance of the water-insoluble GA, liposomes based on PEG-7 glyceryl cocoate were prepared. These PEG-modified GA liposomes showed more favourable particle size and morphology, encapsulation efficiency and stability than the non-modified liposomes. They were also found to display good drug deposition on the epidermis during an *in vitro* study [25].

Other innovative systems supporting transepidermial delivery are nanoemulsions: these are small sized, with high kinetic stability, low viscosity and optical transparency. Their use seems to favor the local administration of active ingredients that have little ability to pass through the skin [26]. Nanoemulsion systems offer greater percutaneous permeability for GA than control O/W emulsion containing the same amount of the active agent. The very small particle size of the nanoemulsions seems to play a crucial role in

Table I Application of GA in various skin disorders and mechanisms of action

Skin disorders	Mechanism of action by GA	Ref.
Hyperpigmentation	Inhibition of tyrosinase	[30]
	Melanin dispersion	[31]
	Acceleration of epidermal turnover	
Atopic dermatitis	Anti-inflammatory action:	[47]
	• Inhibition 12-O-tetradecanoylphorbol-13-acetate-induced glucose transport – reduction of oedema	
	Decrease the secretion of inflammatory mediators:	[50]
	• NO, PGE2, TNF-R, IL-6, IL-1β – reduction of erythema	
	inhibition of leukotriene (LT)B_4 – reduction of pruritus	[38]
Excess of fat	Inhibition of 11β-hydroxysteroid dehydrogenase type 1 at the level of fat cells	[39]
Androgenetic alopecia	Inhibition of dihydrotestosterone (DHT) formation	[41]
Skin ageing	Antioxidant activity:	[53]
	• Increase of the expression of antioxidant enzyme HO-1 • Inactivation of free radicals generated by ultraviolet radiation • Inhibition of lipid oxidation	
	Decrease of the expression of matrixmetallo-proteinase-1 (MMP-1) and -3 (MMP-3)Anti-inflammatory action:	[11]
	• Inhibition of the expression of inflammatory cytokines such as IL-6, TNF-α and IL-10	
Acne	Inhibition of 5-lipoxygenase – reducing of lipogenesis in sebocytes	[51]

increasing the interfacial area of the encapsulated compound into the aqueous phase. Unfortunately, the nanoemulsion did not maintain its stability after five weeks of storage at room temperature: the droplets in the emulsion grew [21].

Applications of 18β-glycyrrhetinic acid for skin disorders

GA itself and licorice root extract, which contains GA as a key ingredient, can be used for the treatment of atopic dermatitis, pruritus and acne vulgaris, as well as the adverse effects associated with sunburn, such as erythema and pigmentation. Its application in dermatology is closely related to its anti-inflammatory and antimicrobial properties [27,28]. GA also is used in the pharmaceutical and cosmetic fields as a lenitive and anti-reddening agent, and is characterized by a good skin tolerability: it is very rare to observe allergic contact dermatitis associated with glycyrrhetinic acid [1,29] (Table I).

GA is a known tyrosinase inhibitor, and has the ability to reduce UVB-induced erythema and pigmentation when applied at topical concentrations of 0.5%. Due to its hypopigmentation properties, it can be successfully used in whitening cosmetic formulations [30]. It is also known to facilitate brightening through melanin dispersion and accelerating epidermis turnover. Topical application of a cosmetic formula containing GA and B-Resorcinol improved skin tone and reduced hand hyperpigmentation when applied to solar lentigines. Previous use of fractional laser photothermolysis did not influence the effectiveness of these two agents [31].

18β-glycyrrhetinic acid has demonstrated low cytotoxicity against normal human keratinocytes, with around 80% cell survival observed at concentrations lower than 30 μM. GA was able to protect human keratinocyte cultures from UVB radiation damage and can prevent oxidative DNA fragmentation and skin cancer induction [32]. GA-d, a derivative produced by the oxidation of the hydroxyl group of GA, was not toxic for HaCaT keratinocytes and human dermal fibroblasts: it increased the proliferation and the migration of both dermal cell lines. In addition, GA-d upregulated the expression of aquaporin-3 in fibroblasts and keratinocytes. These properties make GA a candidate for the treatment of skin diseases like wounds and dermal defects [33].

GA plays a key role against radiation-induced skin damage by a mechanism believed to be associated with its anti-inflammatory activity [11,34].

The efficacy of two formulations (1% and 2%) of licorice extract containing about 20% GA were tested in a double-blind clinical trial for the treatment of atopic dermatitis; the higher concentration (2%) was found to be more effective and reduced erythema, oedema and itching scores [35]. In addition, a multicenter, randomized, placebo-controlled study in adult and children found a hydrolipidic cream with GA, vine leaf extract and telmesteine to demonstrate safe and significant efficacy as nonsteroidal monotherapy in mild-to-moderate atopic dermatitis [36,37].

Topical application of GA has been found to have antipruritic effects. Further *in vivo* research in mice confirmed the efficacy of GA in preventing itching in chronic dermatitis. Although GA reduced cutaneous scratching behavior induced by P substance and protease-activated receptor-2 (PAR-2) agonistic peptide injected to the skin, it did not reduce the scratching evoked by histamine. Its antipruritic effect was most likely based on the inhibition of leukotriene (LT)B_4 production in the skin of the mice [38].

Interestingly, another study on 18 healthy women aged 20–33 years with a normal BMI evaluated the effect of topical application of a cream containing GA (2.5%) on the thickness of fat around the thigh. Both the circumference and the thickness of the superficial fat layer of the thighs were significantly reduced after a month of using the preparation. The activity of the acid was probably related to the 11β-hydroxysteroid dehydrogenase block type 1 at the level of fat cells. It is therefore possible that GA may be used

in the therapy of obesity and in reducing local over-accumulation of fat [39].

The anti-inflammatory activity of GA makes it useful for hair loss treatment. A histopathological study showed that follicular microinflammation plays an integral role in the androgenetic alopecia that is widely recognized as male pattern baldness. In addition, GA inhibits the formation of DHT, which is clearly involved in baldness [40,41].

Anti-inflammatory and antioxidative activity of GA

Inflammation is known to underlie many diseases such as acne [42] and atopic skin [43] but it is also considered a key element of an aging theory called *inflammaging* [44]; briefly, the inflammatory process results in the production of free radicals which intensify the aging process of cells. Assuming this to be the case, the aging process should be slowed by cosmetics containing substances with anti-inflammatory and antioxidant properties.

The structure of GA is similar to those of the mineral-corticoid and gluco-corticoid hormones secreted by the adrenal cortex. The compounds exhibit anti-inflammatory effects by suppressing the expression of pro-inflammatory genes and inhibiting the production of inflammatory cytokines. An *in vivo* study of mice found GA to exert anti-inflammatory activity, by inhibiting 11β-HSD1, and to improve hydrocortisone metabolism within the skin [45]. GA has been used as a topical anti-inflammatory agent to improve the activity of hydrocortisone in skin through the inhibition of 11β-hydroxysteroid dehydrogenase (11β-HSD1), and to induce weak adrenocorticoid-like activity. 11β-HSD1 is a microsomal enzyme which catalyzes the interconversion of cortisone [46].

It has been proposed that GA has more potent effects than glycyrrhizin (GL). It has demonstrated much stronger inhibitory activity than GL with regard to 12-O-tetradecanoylphorbol-13-acetate-induced glucose transport, the substance which triggers oedema in the inflammatory process, as well as stronger binding affinity to mineralocorticoid and glucocorticoid receptors [47].

It has been found that 10^{-4} M GA inhibits 5-lipoxygenase and cyclooxygenase activity. These enzymes activity lead to the transformation of arachidonic acid into pro-inflammatory leukotrienes, such as A4, B4, C4, D4 and prostaglandins [48,49]. Wang *at al.* report that GL and GA reduce the expression of the pro-inflammatory proteins nitric oxide synthase (iNOS) and cyclooxygenase (COX) through the inhibition of NF-kB and PI3K activity [50]. The ability to inhibit 5-lipoxygenase may be valuable in anti-acne medication intended to reduce lipogenesis in sebocytes and acne lesions [51].

Inflammation is a process by which the human organism protects itself from infection with foreign organisms such as bacteria and viruses. Any lack of immune function, or the presence of an immunosuppressive state, can result in opportunistic infections gaining entry to the body.

Steroids exert suppressive effects on the immune system and may cause various side effects associated with the inhibition of keratinocyte and fibroblast proliferation, and of collagen, elastin and glycosaminoglycan synthesis. In addition, many patients suffer from resistance to glucocorticoids. A key advantage of GA over corticosteroids is that while it also displays anti-inflammatory effects, it does not promote the growth of viruses or fungi. This feature of GA was observed in a mouse model of Trichophyton-induced contact hypersensitivity (CHS) [52].

GA can not only activate the glucocorticoid receptor (GR) but also reverse the development of glucocorticoid resistance induced by ROS. To improve GR activation by this compound used to RU486, a glucocorticoid receptor antagonist. GA can activate the intracellular antioxidant system by increasing the expression of the antioxidant enzyme HO-1 [53]. Antioxidant activity is generally directed towards inactivating the free radicals generated by UV light, inhibiting lipid oxidation, and providing protection against UV radiation.

GA has been found to offer protection against UV-induced oxidative damage. Dorsally depilated mice were regularly exposed to UV irradiation over the course of ten weeks, with a group being with treated with topical GA for two hours prior to exposure. It was found that GA treatment increased the activities of key antioxidant enzymes, such as SOD and GSH-Px, while decreasing those of matrixmetalloproteinases MMP-1 and MMP-3 and of various inflammatory cytokines, i.e. IL-6, TNF-α and IL-10 [11].

In addition, oral administration of GA at doses of 100 mg kg^{-1} day has been found to reduce lipid peroxidation and increase antioxidant status in trials with diabetic rats [12].

However, long-term oral administration of GA at high doses has been associated with pseudoaldosteronism, characterized by various adverse clinical effects, including hypertension and hypokalemia [54]. Therefore, topical administration to the skin seems preferable.

Antimicrobial activity of GA

The growth of MRSA and other multidrug-resistant microorganisms represents a serious problem in treatment, and there is an urgent need to develop new antibacterial agents to address this threat. The most commonly-observed nosocomial pathogen is *Staphylococcus aureus*, which is known to cause diseases with high morbidity and mortality. GA demonstrates considerable bactericidal activity against methicillin-resistant *S. aureus* (MRSA) and might act synergistically with some antibiotics [55]. In this case, its activity may be associated with a reduction of the expression of the MRSA virulence genes, including *hla* and *sae*R [56]. The addition of GA strengthens the activity of some antibiotics against strains of methicillin-resistant *S. aureus* (MRSA). The synergistic effect was observed for the combination of GA with aminoglycosides (tobramycin, gentamicin and amikacin) or with polymyxin B on the strain MRSA LUH14616. Antibiotic activity was enhanced 32- to 64-fold for tobramycin, 4- to 8-fold for gentamicin and 8-fold for tobramycin, and 16-64 fold for polymyxin B, when administered in combination with 20 μM of GA. However, no such synergy was observed against LUH14616 when GA was combined with antibiotics from other structural classes, such as cefuroxime, amoxicillin or methicillin, doxycycline, ciprofloxacin or erythromycin [57]. Similarly, GA and its derivative, disodium 3-succinyloxy β-glycyrrhetinate, improved the action of gentamicin against clinical *S. aureus* strains. No such effect was observed for the combination of GA with chloramphenicol, ofloxacin or erythromycin [58]. GA and its 30-piperazine analogue also displayed a synergistic effect when combined with the first-line anti-tuberculous drugs isoniazid, rifampicin and streptomycin; they also significantly reduced the MIC values of these drugs against drug-resistant *Mycobacterium bovis* [59].

GA and its derivatives have been found to demonstrate substantial activity towards various bacterial strains, but not so much against fungi. For example, against several dozen hospital strains of *Staphylococcus aureus* and *Actinobacillus actinomycetemcomitans*, GA demonstrated MIC (minimum inhibitory concentration) values of 64 and 8 μg ml^{-1}, and MBC (minimum bactericidal concentration)

Table II Antimicrobial activity of GA against bacterial strains and fungi

Substantial activity towards	Lack of activity towards
Methicillin-resistant *Staphylococcus aureus* (MRSA)	*Escherichia coli*
Staphylococcus epidermidis	*Proteus vulgaris*
Bacillus subtilis	
Staphylococcus aureus	*Candida albicans*
Actinobacillus actinomycetemcomitans	*Trichosporon beigelii*
Mycobacterium bovis	*Saccharomyces cerevisae*
Periodontpathogenic and capnophilic bacteria isolated from periodontitis	
Pseudomonas aeruginosa	
Helicobacter pylori	

values of 64 and 16 μg ml^{-1} [60]. The MIC values of GA tested against *Bacillus subtilis* and *Staphylococcus epidermidis* were 7.6 and 12.5 μg ml^{-1}, respectively, with no haemolysis of human erythrocytes observed at concentrations up to 100 μg ml^{-1}. Although GA inhibited DNA, RNA and protein synthesis within the bacteria, it did not cause membrane disruption. Interestingly, GA did not demonstrate any antimicrobial activity against *Escherichia coli* or *Proteus vulgaris*, or against the fungi *Candida albicans, Trichosporon beigelii* or *Saccharomyces cerevisae* [61]. Additionally, GA was effective against periodontopathogenic and capnophilic bacteria isolated from adult periodontitis [62]. Subsequent research found GA to demonstrate antibacterial potential against *P. aeruginosa* and to reduce biofilm formation and the secrete virulence of opportunistic nosocomial *Pseudomonas aeruginosa* ATCC 25619 [63].

GA also has a beneficial influence on peptic ulcers, which has been associated with its strong *in vitro* activity against 29 different *H. pylori* strains. Its effectiveness against clarithromycin-resistant strains provides hope that GA can serve as an alternative therapeutic agent against *H. pylori* [64] (Table II).

To enhance the antibacterial activity of GA and minimize its side effects, a PLGA (poly lactide-co-glycolide) based nanocarrier system was developed. GA-loaded PLGA nanoparticles have demonstrated greater activity against *S. aureus, S. epidermidis* and *P. aeuroginosa* than GA alone, which may be due to the greater penetration of the nanoparticles into infected cells [65]. Another way to improve the activity of a compound is by structural modification. The substitution of 18β-glycyrrhetinic acid with hydroxyl groups in the C-1 and C-2 positions enhanced inhibitory activity against Gram–positive bacterial strains (*S. scabies, B. subtilis, S. aureus,* MRSA). These derivatives are believed to influence the regulation of the expression of the genes associated with peptidoglycan production, the respiratory metabolism and the inherent virulence factors found in bacteria [14].

Conclusion

18β-Glycyrrhetinic acid is characterized by not only a wide range of valuable biological properties but also by interesting dermatological applications.

From the point of view of dermatology and cosmetology, its most significant asset is its anti-inflammatory efficacy, which is particularly important as inflammation processes form the basis of many skin concerns, such as acne, atopic skin or ageing skin. GA exhibits anti-inflammatory activity by suppressing the expression of pro-inflammatory genes, inhibiting the production of inflammatory cytokines and influencing the transformation of arachidonic acid into pro-inflammatory leukotrienes. Although its structure is similar to that of hormones secreted by the adrenal cortex, it does not promote the growth of viruses or fungi. Additionally, GA has antioxidant properties and this activity is directed to the inactivation of free radicals and inhibition of lipid oxidation. GA displays low toxicity against normal human cell lines, fibroblasts and keratinocytes.

Its substantial antimicrobial activity towards some strains of bacteria indicate that GA could be promising candidate for the treatment of microbial-inducted diseases and skin disorders. In addition, it is worth noting that GA has an ability to strengthen the activity of some antibiotics towards MRSA: tobramycin, gentamicin, amikacin and polymyxin B. GA could be added to cosmetic formulations to not only support the primary functions of the active substance, but also to perform auxiliary functions as a preservative.

Acknowledgements

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