

**Review Article**

**AN OVERVIEW ON THE BIOLOGICAL AND PHARMACOLOGICAL ACTIVITIES OF SAPONINS**

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**ABSTRACT**

Saponins are secondary metabolites synthesized by many different plant species and marine animals. They derive their name from the Latin word "sapo" meaning soap, due to their surfactant properties, which allows forming stable soap-like foam when shaken in aqueous solution. They are large molecules and contain a hydrophobic part, composed of a triterpenoid (30 carbon atoms) or steroid backbone (27 carbon atoms with a 6-ring spirostane or a 5-ring furostane skeleton) and a hydrophilic part consisting of several saccharide residues linked to the hydrophobic scaffold through glycoside bonds. They have many medical uses including microbial, anti-tumor, anti-insect hepatoprotective, haemolytic and anti-inflammatory activities. They also decrease the blood cholesterol level and may be used as adjuvant in vaccines. In addition, saponins are used in preparation of soaps, detergents, fire extinguishers, shampoos, beer and cosmetic. Many saponins that exhibit haemolytic activity have a bitter taste and are toxic to fish. This work provides an overview on the biological and pharmacological activities of saponins with a special focus on their mechanism of action.

**Keywords:** Saponins, Biological activities, Pharmacological activities.

**INTRODUCTION**

Saponins are a group of secondary metabolites, nonvolatile surfactants that are widely distributed in the plant kingdom and marine animal [1]. This group of natural products has the same common characteristic of foaming when shaken with water. The formed foam is a result of the formation of a colloidal solution, having a stable and lasting action of dilute mineral acids, differing from ordinary soaps. Another important feature of this class of substances is related to the ability of precipitating cholesterol by forming insoluble complexes. Both features are correlated to the above amphiphilic or amphiphilic nature of these molecules, since they are formed by one hydrophilic and one lipophilic and one lipophilic moiety [2].

Saponins have a range of properties due to their extensive structural diversity, which includes certain features, bitter sweeteners, detergents and emulsifying properties, in addition to the biological, medical and pharmacological properties, such as haemolytic activity, antimicrobial, insecticides and molluscicides. Also noteworthy are the applications in pharmaceutical industries as raw material for the synthesis of steroidal drugs such as birth control, besides the intense use in the cosmetic industry [3].

The usage of plants containing saponins was accomplished by early civilizations because of their detergents to fish and toxic properties. The early study of biological activities of this class of substance was conducted through extracts rich in saponins. Currently, the occurrence in nature, the ethnopharmacological use and the various biological activities of saponins, scientifically proven, have aroused the interest of mankind and, especially, of the scientists worldwide.

However, the saponins are highly toxic when administered intravenously in higher animals, despite presenting a multitude of biological activities. This toxicity falls to low levels when administered orally and, additionally, some saponins present in food do not offer oral toxicity significantly [4].

The amphiphilic behavior of saponins and the ability to form complexes with steroids, proteins and membrane phospholipids determined a number of different biological properties for these substances, especially the action on cell membranes, changing their permeability, or causing their destruction. Related to this action on membranes the haemolytic, ictiotoxic, spermicide and molluscicidal activities are frequently observed. The complexation with cholesterol yielded a significant number of studies in order to

evaluate the use of saponins in the diet to reduce serum cholesterol levels, antihypercholesterolemic activity. The anti-inflammatory and antiallergic activity of saponins has been known for a long time and is closely related to the core aglycone.

Numerous papers have been published reporting the activities of saponins, among the most impressive antidiabetic, antifungal, antitumor activity, antiviral, antiparasitic, immunomodulatory, synthesis of hormones, acting on the cardiovascular system, acting on the central nervous and endocrine systems.

Furthermore, the saponins are important for many actions of drugs derived from plants, such as diuretics, expectorants and components. However, the mechanism of these activities have not been fully elucidated, it is believed that the irritation caused in the respiratory and renal tract epithelia contribute significantly to the observed activities. Saponins are also widely used in the pharmaceutical industry as adjuvants to enhance absorption of other drugs by increasing solubility or interfering in the mechanisms of absorption [5].

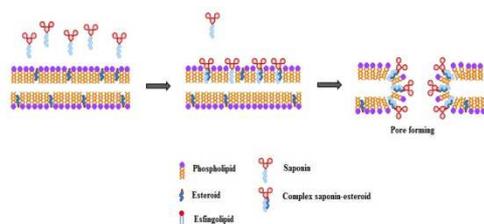
**Haemolytic activity**

Chemical studies and analyzes of cell membrane permeability show that the composition has lipoprotein or are formed by lipids and proteins. Phospholipids have in their molecules a hydrophobic end with aversion to water and another hydrophilic end with affinity for water. The plasma membrane has a biomolecular layer of lipids with the hydrophobic portions of molecules facing each other, and facing internal and external membrane surface hydrophilic moieties. Dipped in "mat fat" are the protein molecules, now exposed on the outer face, in the inner face or sometimes on both sides of the membrane.

The ability to cause perturbation on the membrane is the most widely studied effect of saponins, especially the steroidal saponins. This property is often associated with haemolytic capacity of saponins to cause lysis of erythrocytes in mammals. The mechanism of action of these secondary metabolites, that would be the cholesterol target, is essential for the formation of membrane pores. Since then, many research groups have tried to elucidate the molecular basis of this activity by studying the fragments of saponins. The results confirmed the influence of the chemical composition of the membrane, especially regarding the concentration and structure of sterols in the permeability change when saponins are added. Therefore, it becomes obvious the

haemolytic potential of saponins is affected by its chemical characteristics, including the structure of aglycone, the chain number of sugars, the long chains as well as the types of sugars and incorporated binder units of sugar.

Glauert *et al.*, 1962 proposed the first model for the mechanism of action of saponins on the membranes. According to this model, the spontaneous formation of complexes between saponins and cholesterol in the membranes is followed by association with a micelle, two-dimensional structure within the membrane. The hydrophilic sugar chains are oriented towards the center of the micelle complex, leading to the formation of an aqueous pore that, in turn, would cause an increase in membrane permeability allowing the passage of ions and macromolecules across the lipid bilayer (Fig. 1).



**Fig. 1: Model of the mechanism of action of saponins on the membranes.**

Newer models have expanded the initial hypothesis Glauert *et al.*, 1962. The first step of this mechanism of action is the incorporation of saponins to the membrane through its steroidal nucleus. This incorporation occurs spontaneously due to the lipophilic characteristics of the aglycone and the hydrophobic characteristics of the membrane sterols forming a complex saponin and accumulating cholesterol in the matrix or membrane plates. On the surface, it is noticeable a core carbohydrate cluster by changing the surface tension and causing a curvature of the membrane as the final step of the perturbation mechanism.

This curvature may result in pore formation, lysis of erythrocytes or tubular protuberances that may eventually lead to blistering via extraction of sterols, which explains the low haemolytic capacity of some saponins. An alternative recent model attempts to explain the disruption of the membrane domain observed by some groups of saponins. In this model, after the integration of the saponin to the membrane, the aglycone of saponins migrate toward areas of membrane sphingomyelin and complex cholesterol. Complex formation interferes with the specific features of the domain, resulting in its rupture. Similarly to the last model, the accumulation of saponins in membrane domains has been the cause of curvature of the membrane dose-dependent manner [1].

In most cases, steroidal saponin or steroidal glycoalkaloids have higher haemolytic activity of triterpenoidals saponins, since the steroidal core has a higher affinity for the erythrocyte membrane cholesterol due to a greater structural similarity. The haemolytic activity is inhibited when cholesterol is added in the middle of the reaction, confirming the involvement of interaction between saponin and cholesterol membrane in hemolysis [7].

#### Molluscicidal activity

Some saponins have shown molluscicidal activity against the snail *Biomphalaria glabrata*, intermediate host of *Schistosoma mansoni* parasite that causes schistosomiasis that affects millions of people in Asia, Africa and South America.

The monodesmosidic saponins containing the sapogenin oleanolic acid are those with the highest activity, because these saponins interact with greater intensity with the cholesterol present in cell membrane altering their permeability, promoting the formation of pores and allowing leakage of liquids. This same mechanism of action can be used to explain the activities and ictiotoxic spermicides presented by some saponins [8].

The eradication of the intermediate hosts and chemotherapy are the only ways to control schistosomiasis. Currently, the main molluscicide used is niclosamide. However, the high cost makes their use impractical for populations where schistosomiasis is endemic, besides being toxic to humans. Thus, due to the high toxicity of saponins for molluscs and their low toxicity for humans when orally ingested, the substances produce good candidates for development of an alternative chemotherapy to combat the causative vector of schistosomiasis [9].

#### Anti-hypercholesterolemic activity

Hypercholesterolemia is a risk factor that contributes to the development and progression of atherosclerosis and subsequent cardiovascular disease. Epidemiological and clinical data have shown that high concentrations of LDL cholesterol in the bloodstream is the large pivot of these diseases [10]. Bioactive compounds with hypocholesterolemic activity have been conducted, among the most studied are soluble fibers, phytosterols, phospholipids, soy protein, stearic acid and saponins [11]. There are several mechanisms which siphoning may reduce cholesterol levels:

- (1) Formation of an insoluble complex where it is added to the beta-hydroxysteroid, thereby decreasing intestinal cholesterol absorption, producing an increase of sterols which are excreted along with feces;
- (2) Adsorption of bile acids in the diet of the fibers is increased in the presence of saponins because they form micelles with large molecular weights, which prevent bile acids that are reabsorbed. Thereby the increase occurs in the liver through the conversion of cholesterol into bile acids;
- (3) Interaction with cells of the intestinal mucosa promoting a higher permeability of these cells and subsequently a rapid loss of cell function by increasing proliferation promoting exfoliation and loss of this function. Thus, it contributes to a further increase in the excretion of cholesterol;
- (4) The presence of sugars,  $\beta$ -1, 4 connected enhances the absorption of soluble fiber and promotes the reduction of fatty acids, resulting in a decrease of liver cholesterol [12].

#### Anti-inflammatory and antiallergic activity

The evaluation of anti-inflammatory activity of saponins have been performed using models of inflammation with carrageenan [13]. In general, the oleanane and ursan sapogenins are those with higher activity. The mechanisms considered for this activity include corticoidmimetic activity inhibiting the degradation of the glucocorticoid of release of mediators of inflammation, inhibition of enzyme formation and inhibition of increased vascular permeability [14].

#### Cytotoxic and antitumor activity

There have been numerous reports of scientific papers in relation to the cytotoxic properties of saponins, however saponins do not always have high cytotoxic antitumor properties. The cytotoxic mechanism of saponins occurring via inhibition of DNA synthesis induces a reverse phenotypic transformation into tumor cells. When the antitumor mechanism occurs through the inhibition of vessels around the tumor, there is an inhibition of tumor growth. Inhibition of metastasis, as well as immunostimulation and observed chemoprevention mechanisms are important in antitumor compounds. As almost all saponins induce apoptosis in tumor cells, they become the preferred drug in treating cancer because they eliminate tumor cells with low side effects for the patient, avoiding mainly necrosis [15].

#### Antiviral activity

Some saponins may inhibit DNA synthesis of the herpes simplex virus, such as those with sapogenin of the oleanane type, whereas sapogenins type ursan inhibit the synthesis of viral capsid protein of the same virus. Other saponins may also inhibit virus type II polio through inhibition of the attack of the virus to the host cell. These compounds have been shown to reduce experimental keratitis caused by herpes simplex virus in rabbits [16]. Saponin obtained

from *Glycyrrhiza glabra*, glycyrrhizin, is the main representative of biological activity. It promotes the inhibition of virus comprising DNA and RNA, including HIV-1 in vitro. This process occurs via inhibition of the protein kinase C, which works to link these viruses to CD4+ receptors present on T cells and to inhibit the enzyme reverse transcriptase [15].

#### Anti-diabetic activity

Many natural products are used in folk medicine as hypoglycemic. Some of them have been isolated, identified and their activities have been evaluated by in vivo testing in animals with induced diabetes. There are several mechanisms by which saponins exert their effects, among the most important we can mention the inhibition of adrenocorticotropin-induced, suppression of lipogenesis activated by insulin which leads to their increase, increased glycolytic activity, decreased gluconeogenesis and lipolysis synthesis r-RNA and m-RNA, which act on the metabolic regulation in diabetic rats [15].

#### Antifungal, antiparasitic and antibacterial activity

The interest in new antimicrobial agents from plants has been restored during the last 20 years, as traditional antibiotics (mainly those derived from microorganisms) are ineffective produce several side effects and favor the development of resistant strains [17]. The antifungal, antiparasitic and antimicrobial activity of saponins can be observed by the ability to form complexes with cholesterol membrane resulting in an increase in permeability and consequent leakage of cytoplasmic contents [18].

#### Hormone synthesis

Steroidal saponins found in the rhizomes of several species of the genus *Dioscorea* have a steroidal content around 40% and 50-60% of Diosgenin and other sapogenins [19]. Diosgenin is treated with acetic anhydride and ester forms a pseudosapogenin. Then when oxidized with chromium trioxide followed by hydrolysis forms two intermediate compounds and finally progesterone (Fig. 2) [20]. This work, of crucial importance for the development of steroid therapy, contributed significantly to the subsequent development of the female contraceptive pill. From this, many different classes of natural products have been used as raw material for the synthesis of different bioactive substances. The steroidal glycoalkaloids isolated from the leaves of tomato plants, as tomanidin another example of usage in the synthesis of steroid hormones [21].

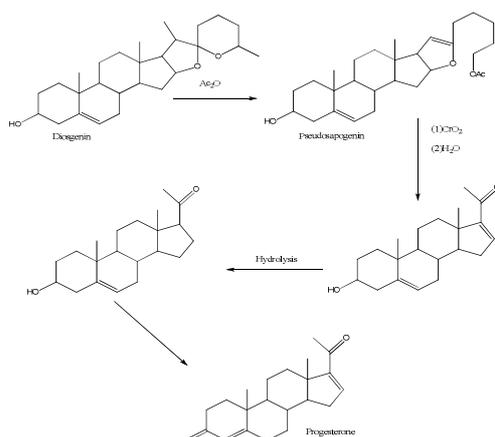


Fig. 2: Synthesis of progesterone from Diosgenin

#### Acting on the cardiovascular system

There may be two different types of action of saponins in the cardiovascular system: the heart action (contractile force, automaticity, rhythm, effect of lipid peroxidation) and effects on the properties of the blood (clotting).

#### Cardiac activity

A mixture of triterpenoid saponins from *Dianthus gypsophila* was capable of producing a positive inotropic effect on cardiac muscle in

the ferret at a concentration of 0.025 mg/ml and 0.05 mg/mL. In the presence of normal concentrations of extracellular calcium, to prevent exfoliation, a saponin mixture produced an increase in the levels of intracellular sodium and potassium levels decreased not only due to inhibition of the sodium-potassium pump. The changes in the levels of intracellular ions were accompanied by contraction. Thus, the effects of saponins could be explained by interaction with cholesterol, which results in increased membrane permeability to sodium. The saponins of ginseng have shown significant cardiovascular effects. The Ginsenosides Rb1, Rb2 and Rb3, 30µg/mL restored the action potential of the Wistar rat cardiomyocytes damaged by free radicals, indicating an antioxidative action. In cardiac cells, these same saponins, 20µg/mL, inhibited the action potential and contractility, suggesting a possible blockade of calcium channel [15].

Recent studies demonstrate that ginsenoside Rb3 has decreased significantly, the myocardial injury induced by isoproterenol in 5-15mg/mL dose in rats, confirming the cardioprotective activity of this saponin. Isoproterenol has positive inotropic and chronotropic effect and is an agonist of β<sub>2</sub> adrenergic receptor, causing necrosis of the myocardium [22].

#### Action on blood clotting

Some saponins inhibit in vitro the aggregation induced by aggregating agents (endotoxins, collagen, arachidonic acid, adenosine diphosphate) platelets. Some observations on the mechanism of action have been made, such as increase in cAMP levels in platelets, decreased production and release of thromboxane (TBX) and inhibition of the production of prostacyclin (PGI<sub>2</sub>). Ginsenoside R0 inhibited the conversion of fibrinogen into fibrin, induced by thrombin at concentrations of 0.1-1.0 mM, since the Rb1, Rb2, Rb3, Rg1 and ginsenoside Rg2 promote the action of urokinase that activates the conversion of plasminogen into plasmin, which, in turn, degrades the fibrin network [15].

#### Action on blood pressure

In anesthetized rats, ethanolic extract of *Symphytum officinale* and a saponin hederagenin caused a systolic blood drop and the diastolic blood pressure dose-dependent manner. However, there is no mechanism established for such hypotensive effects [15].

#### Action on Central Nervous System

Ginseng saponins have shown biomodulatórios effects on higher centers of the central nervous system, facilitating the physical and mental activities. They also have anti-stress activities and effects on central neurotransmitters in hypobaric hypoxia. The results demonstrate that ginsenoside alter the effects of barbiturates and convulsants, suggesting that the GABAergic neurotransmitter regulation may have important pharmacological actions of ginsenosides. This has been proven by many studies that have confirmed the facilitation of saponins of ginseng on learning and memory. Other saponins have shown sedative and analgesic activity, such as saponins *Aster batangensis*, contrary to amphetamine and synergistic to the chlordiazepoxide effects [15].

#### Action on the endocrine system

Triterpenoid saponins from *Tetrapleura tetrapterain* inhibited the release of luteinizing hormone (LH) in a dose-dependent culture of pituitary cells so. This explains the inhibition of anti-gonadotropic extracts of *T. tetraptera*, which are used as natural contraceptive properties [15].

#### Immunoadjuvant activity

The term adjuvant means support and is defined as a substance that, when incorporated into a vaccine formulation, accelerates, prolongs and enhances the quality of specific immune responses. For data, such as aluminum phosphate and aluminum hydroxid, they are the only immunologic adjuvants approved by the FDA. Nevertheless new adjuvants have replaced aluminum, which are often more effective than it, increasing the number of antibodies and cellular responses. Some of these new adjuvants demonstrated ability to increase the immunogenicity of vaccines against infectious diseases and cancer.

The adjuvant vaccine has several advantages because they increase the performance of vaccines, enhance the immunogenicity of weak immunogens through purification and recombinant antigens, reduces the amount of antigen or the frequency of immunizations required to promote adequate protection, increases the effectiveness of vaccines in infants and immunocompromised adults and promotes T cell proliferation and cell-mediated immunity. The benefits of incorporating adjuvants in vaccine formulations to enhance immunogenicity must overcome the risks once these agents may cause adverse reactions. Local adverse reactions include injection site inflammation or, rarely, induction of granulomas. Systemic reactions observed in laboratory animals include pain, fever, arthritis and others. The reactions can be caused by the interaction with the antigen or adjuvant due to the type of response or even the type of cytokine. Thus, an extensive study of the toxicity of these adjuvants is to be performed, as well as the formulation of the vaccine, to ensure safety of the candidate vaccine for testing in Phase 1 through conducted clinical trials [23].

The first saponin adjuvant was obtained from the tree *Quillaja saponaria*. It is known as saponin QS-21 and consists of a core of triterpene aldehyde and two sugar chains, one of which is acylated by means of ester bond (normally monoterpenes) and lipophilic aliphatic acids bound fucose. The stimulation of the Th2 response is related to the balance of the hydrophobic and hydrophilic respectively represented by the core triterpene and the sugar chains. While stimulation of Th1 response is related to the presence of structures acylated this chain, acylated normal monoterpenes, for performing the deacylated saponin from this, it appears that there is loss of stimulation of the Th1 response, but the Th2 is maintained. A major distinguishing feature of this saponin is a costimulatory signal Th1 response through the aldehyde-grouping present in the aglycone thus facilitating the production of CTL against exogenous antigens [24].

#### CONFLICT OF INTERESTS

Declared None

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