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Review article

# Cardiotonic effects of cardiac glycosides from plants of Apocynaceae family

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Cardiac glycosides (CGs), such as digoxin, were one of the earliest pharmacological treatments used clinically in the management of heart failure. Plants from the Apocynaceae family which grow indigenously in Asia especially in the tropical and subtropical regions and one of the largest plant families, are producers of CGs. The purpose of this review is to update the research progress of CGs with cardiotonic effects obtained from Apocynaceae family on heart failure. Heart failure increases morbidity and mortality globally. It is manifested by compromised contractility of the heart to pump adequate blood and oxygen to all other parts of the body. CGs bind to $Na^+/K^+$-ATPase and inhibit the sodium pump, which is believed the major mechanism of action that contributes to its cardiotonic properties. The mechanisms of action of various CGs, particularly the cardenolides present in Apocynaceae plants are discussed in this article. Besides digoxin, other CGs have the potential to be developed as an alternative or adjunct therapy for the management of heart failure.

***Keywords:*** Oleander, oleandrin, neriifolin, sodium pump, Apocynaceae.

There are many modern drugs available for the treatment of heart failure. One of the oldest is CGs, such as digoxin and ouabain.[1] These therapeutics have positive inotropic or cardiotonic effects on the heart, improving heart pumping capacity. Ouabain is isolated from *Strophanthus gratus* of the Apocynaceae family.[2]

The family, Apocynaceae, commonly known as the oleander or dogbane family, ornamental plants, has roughly 200 genera and 2,000 species of angiosperm plants, including *Adenium obesum, Allamanda catharttica, Alstonia macophylla, Alstonia scholaris. Alyxia reinwardtii, Carissa spinarum, Catharanthus roseus, Cerbera manghas, Cerbera odollam, Holarrhena antidysenterica, Holarrhena curtsii, Nerium oleander, Plumeria obtuse, Plumeria rubra, Rauvolfia serpentine, Thevetia peruviana* and *Wrightia tomentosa*.[3] They are flowering plants commonly found in tropical and subtropical regions, especially in the Asia. A few plant parts from these are well-known for their alkaloids and CGs, which can either be medicinal or poisonous depending on the dose and type of chemicals present.[4]

Heart failure is a chronic condition that normally affects older populations. It may occur due to many factors such as cardiac overload or post-myocardial infarction. It is initially manifested by the enlargement of the heart due to remodeling, clinically known as cardiac hypertrophy, owing to an adaptive mechanism to compensate hypertensive states. Later, this pathological condition progressively results in the development of heart failure. It is characterized by a diminution in cardiac output based on impairments in blood pumping capacity. Therefore, the heart is unable to supply blood and oxygen adequately to the peripheral tissues to meet their metabolic demands.[5]

The pathophysiology of cardiac hypertrophy is multifactorial, which includes upregulation of fetal genes, histopathology and cardiac dysfunction. It is now clear that hypertrophy events are mediated by distinct signaling molecules. Therefore, research related to heart failure focuses on identifying and inhibiting pathological processes.[6]

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***Cardiac glycosides from the Apocynaceae family***

The CGs' chemical structure comprises aglycone (steroid-like moiety with lactone ring) and glycone (sugar moiety). There are two types of CGs - cardenolides and bufadienolides based on the differences in the lactone rings.[3] The genera of *Nerium*, *Cerbera*, *Thevetia*, *Strophanthus* and *Apocynum* are among the notable sources of natural cardenolides from the Apocynaceae family. Generally, cardenolides are more diverse when compared to bufadienolides. The aglycone structure of CGs consists of a steroid moiety with an unsaturated lactone group attached at position C-17. The aglycone is combined with glycone (a sugar moiety) at its C-3 position (Figure 1). The steroidal framework is considered the pharmacophoric core responsible for the medicinal or biological activity of these compounds.[7]

Glycoside refers to the attachment of the sugar moiety to the steroid core. The glycone moiety (sugar) itself may not exert any medicinal activity. However, the combination of certain glycones and aglycones may influence the pharmacodynamic and pharmacokinetic activities of each CG. The potency of CG compounds is influenced by the type of the attached sugar, as in the case, for instance, with the addition of rhamnose that shown to increase potency several times (six to 35 times), whereas the addition of mannose had no significant impact on CG potency.[8] In the Apocynaceae family, the sugar moiety comprises a wide variety of sugar residues such as rhamnosyl, acofriosyl, oleandrosyl, thevetosyl, digitalosyl, sarmentosyl, diginosyl, 6-deoxy-allosyl. glucosyl, gentiotriosyl and gentiobiosyl.[7] As mentioned previously, the lactone group attached to aglycone characterizes whether the CG is a cardenolide or bufadienolide (Figure 2). Cardenolides have an unsaturated five-membered lactone ring, whereas bufadienolides have an unsaturated six-membered lactone ring, both located at C-17[4] (Figure 1). Examples of cardenolides with cardiotonic property isolated from the plants of the family Apocynaceae are ouabain[9], oleandrin[10] and tanghinin.[11]

***Pharmacokinetics of CGs***

To date, not much published data on the pharmacokinetics of CGs especially the cardenolides from Apocynaceae. Due to the difference in the chemical structure, the pharmacokinetic profile varies among the cardenolides. In humans, oral absorption of the cardenolides from *Thevetia peruviana* follow zero order kinetics and their 24 h-area-under-the-curve ($AUC_{24}$) is 19.0 µg.h/L. These cardenolides reach median peak concentration ($C_{max}$) at about 1.05 µg/L after 12.5 h (median $T_{max}$).[12] Oleandrin on the other hand, achieves $C_{max}$ at 20 min after oral administration in mice. Its oral bioavailability is about 30.0%.[13]

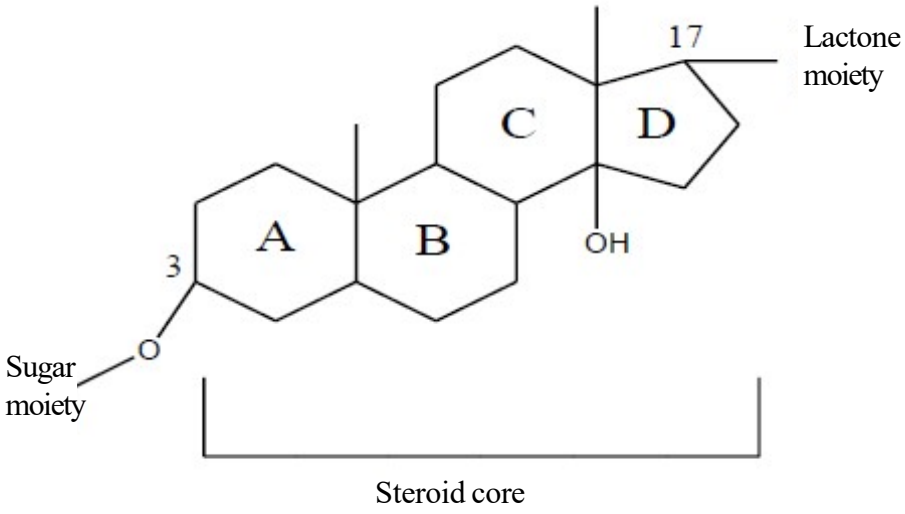


**Figure 1.** The basic structure of cardiac glycosides.

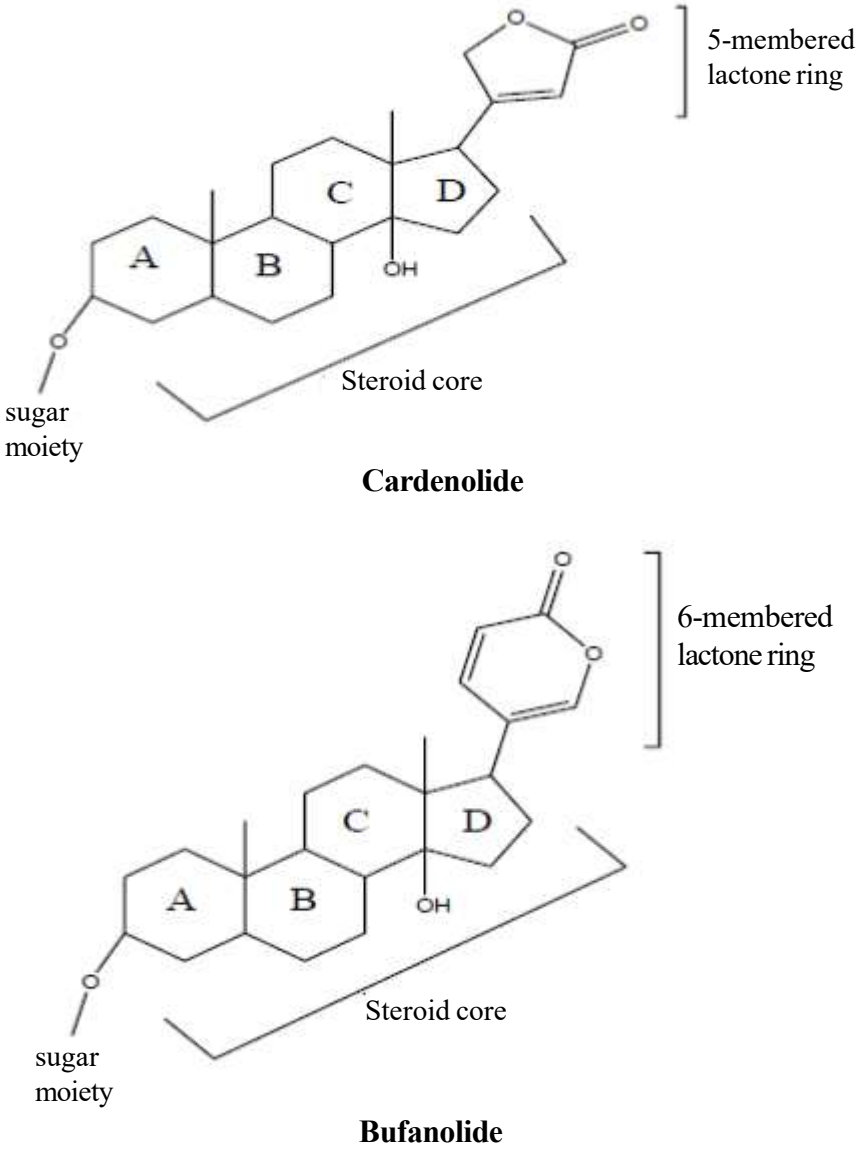


**Figure 2.** The basic chemical structure of glycoside cardenolides and bufalinolides.

Oleandrin is distributed to the liver, kidney, heart and brain.[13] On repeated dosing via intravenous route, ouabain reaches steady-state plasma concentration after 4 - 5 days. The drug has biological half-life of about 18 hours in dogs and 21 hours in humans [14], while the apparent terminal half-life of the *Thevetia* cardenolides is 62.9 h.[12] The elimination half-life of oleandrin after oral dosing is about 2.3 h.[13]

Metabolism has different effects on the cardenolides. Glucosidation decreases uzarigenin activity but increases the activity of digitoxigenin. On the other hand, conjugation with rhamnose enhances the activities of both cardenolides.[15] Excretion of other cardenolides is not clearly known. However, ouabain has been reported to undergo renal excretion.[14] Oleandrin is also excreted in urine as the parent compound and its metabolite, oleandrigenin, but almost 70.0% of the administered dose is found in feces, comprising both compounds.[13]

### *Pathophysiology of heart failure*

Heart failure occurs when the heart is unable to pump out enough oxygen-rich blood to the body, and it may affect one or both sides of the heart ventricle. It is one of the main causes of morbidity and mortality in the Western world. The prevalence, mortality and costs associated with this disease are still rising, despite the implementation of current recommended therapies for the treatment of the disease.[16]

As stated earlier, myocardial contractility is impaired in heart failure. The force of myocardial contraction is determined by calcium cycling in cardiomyocytes, referring to release and reuptake of intracellular calcium. Calcium levels are the central regulator of myocardial contractility. In failing hearts, calcium cycling is significantly altered. These defects could occur at the storage site of calcium, namely the sarcoplasmic reticulum, or at any of its regulatory steps. Physiologically, calcium is released from the sarcoplasmic reticulum via the action of ryanodine 2. Sarcoplasmic/endoplasmic reticulum calcium ATPase 2a (SERCA2a) then transports back the calcium into the sarcoplasmic reticulum. The level of the calcium is also regulated by other transporters, such as sodium-potassium ATPase ($Na^+/K^+$-ATPase) and sodium-calcium exchanger (NCX). $Na^+/K^+$-ATPase has indirect effects on calcium levels. Physiologically, $Na^+/K^+$-ATPase, also known as the sodium pump, is responsible for pumping three sodium ions out of the cells in exchange for two potassium ions. Increased extracellular sodium ions will promote sodium-calcium exchange by NCX, a major calcium efflux mechanism of ventricular cardiomyocytes. It transports three sodium ions in exchange for one calcium ion, either into the cells or outside depending on its modes. In forward modes, calcium ions are extruded from the myocytes; while in reverse mode, sodium ions are expelled.[17]

### *Cardiotonic effects and mechanism of action of Apocyanaceae CGs*

Ouabain is one of the most studied CGs from Apocynaceae. It has been used clinically and referred to as g-strophanthin in Germany.[9] Other than ouabain, other CGs isolated from the Apocynaceae family have yet to enter clinical trials. Most studies are conducted within an *in vitro* setting. Not many *in vivo* studies have been reported on the effects of the CGs on heart mechanical performance despite their potential impacts.

Ouabain is believed to exert its protective effects against heart failure by binding to and then inhibiting the $Na^+/K^+$-ATPase enzyme.[18] It exerts its inotropic effects via both modulating calcium levels and producing reactive oxygen species (ROS). Its inhibition on the $Na^+/K^+$-ATPase $\alpha$-subunit promotes Src, Ras and mitogen-activated protein kinases (MAPKs), leading to an increase in intracellular calcium.[19] Activation of MAPKs promotes mitochondrial ROS.[20] When ouabain-induced ROS production is blocked, it also partially blocks ouabain-induced contractility.[21] Continuous infusion of this drug at 50 μg/kg/d for 4 weeks is beneficial in cardiac failure, owing to its effects in preventing or slowing down cardiac remodeling via activation of phosphoinositide 3 kinase $\alpha$ (PI3K).[22]

However, the inotropic effects of ouabain are also reported by activation of the $Na^+/K^+$-ATPase enzyme extracellularly before triggering intracellular calcium release via signal transduction pathways.[23] At lower concentrations ($10^{-8}$ to $5 \times 10^{-7}$ mol/L), the drug stimulates the sodium pump in sheep Purkinje fibers, which is dependent on the extracellular potassium concentration. At these stimulatory levels, positive inotropism of ouabain is observed.[24] Lüllmann *et al*.[25] noted that the inotropism of the glycosides is not necessarily associated with sodium pump inhibition.

Crude extract of dried leaves of *Nerium oleander* (2-100 mg/ml) perfusion elevates heart rate, amplitude and force of cardiac contraction in isolated guinea pig hearts.[26] Two CGs, namely neriine and oleandrin, are known to be present in all parts of *Nerium oleander.*[27] Thus, the effects seen with the crude extract of *Nerium oleander*[26] might also contribute to these two cardenolides. It is believed that the main mechanism behind the positive inotropism of cardenolides is via inhibition of $Na^+/K^+$-ATPase activity.[10] Oleandrin (25 μg/ml) exhibits positive inotropic function by enhancing intracellular calcium levels as seen in rat cardiomyocytes via inhibition of ryanodine receptor calcium release channels and $Na^+/K^+$-ATPase.[10] Another compound identified as 17βH-neriifolin from *Cerbera odollam* leaves targets and binds to $Na^+/K^+$-ATPase with inhibitory effects comparable to ouabain when evaluated with a molecular docking approach.[28]

Other than oleandrin, tanghinin and acetyl-tanghinin isolated from *Tanghinia venenifera* (also known as *Cerbera manghas*) also possess positive inotropic activity. These cardenolides increased the *in vitro* contractile force of papillary muscle from guinea pigs (Figure 2). Acetyl-tanghinin exhibited a higher maximal response than tanghinin. However, above the concentration of the maximal response (2.8 μM), their positive inotropic impacts rapidly dropped, and a rise in diastolic tension was seen.[11] No further study was carried out to elucidate the possible mechanism of these cardenolides on heart inotropy.

Extracts from *Apocynum venetum* (1 mg/ml) that contain cymarin, a CG, showed cardiotonic effects in isolated guinea pig atrium. The observed effects were not via $\beta_1$ activation based on no inhibition noted with the addition of propranolol, a β-adrenoceptor antagonist, but via inhibition of the phosphodiesterase-3 enzyme. The enzyme promotes $Ca^{2+}$ influx that increases myocardial contractility by inhibiting the degradation of 3', 5'-cyclic adenosine monophosphate (cAMP).[29] It seems that different CG compounds act differentially when exerting their cardiotonic properties, as summarized in Figure 3. Different cardenolides have different range of therapeutic doses or concentrations due to their small therapeutic indices. Most studies conducted were in *in vitro* and *ex vivo* settings, therefore, more *in vivo* studies need to be performed to carefully estimate the therapeutic range of the individual compounds.

***Potential of toxicity of Apocynaceae CGs:***

CGs are plant-based natural products that may have inherent toxicity when ingested, and many are very toxic, even lethal if taken without medical guidance. Certain parts from the plants of the Apocynaceae family are generally considered extremely poisonous. The genera *Apocynum, Acokanthera*, *Nerium*, *Cerbera*, *Strophanthus* and *Thevetia* have therapeutic properties but are often associated with toxicity, and in many cases are lethal. Alkaloid-producing species like *Rauvolfia*, *Tabernathe* and *Catharanthus* are examples of the source of compounds with potential therapeutic properties but have been associated with accidental poisonings when not taken in appropriate doses.[30, 31] They target the heart failure-causing ventricular arrhythmias.[32]

*Nerium oleander* is highly toxic, owing to the presence of oleandrin and neriine. The former compound reversibly inhibits the α3 subunit of the $Na^+/K^+$-ATPase pump.[13, 33] A recent *in vivo* study by Botelho AFM, *et al*.[34] demonstrated that the oral administration of extract of Nerium oleander at 150 and 300 mg/kg, which primarily contains oleandrin, showed positive inotropic activity in guinea pigs. However, at higher doses, it caused severe cardiac arrhythmias, which might lead to death. As little as 1 oz of plant materials (*Nerium oleander*) can be lethal to a 1000-pound horse.[35] In sheep, clinical signs of toxicosis start to appear approximately after 30 min following oral administration of dried leaf extract of the plant at 110 mg/kg body weight. It causes ruminal atony, abdominal pain, polyuria, pollakiuria, bradycardia and arrhythmia. Electrocardiography has shown S-T segment depression, atrioventricular blocks, ventricular premature beats and ventricular fibrillation.[36] Common adverse effects of oleandrin observed clinically are fatigue, nausea and diarrhea, as well as grade 1 atrioventricular block and grade 2 supraventricular tachycardia monitored by electrocardiography when taken at 0.3383 mg/kg/d for 21 - 28 days in patients with advanced cancer.[37]

Red-flowered varieties of oleander appear to be more toxic, with the fatal blood concentration of oleandrin estimated for humans to be approximately 20 ng/ml.[38] Its mortality is very low in humans, but a single leaf can be fatal to a child eating it. The lethal dose of green oleander leaves for cattle and horses has been found to be 0.05% of the animal's body weight. The minimum lethal dose of oleander for cattle is roughly 50 mg/kg body weight. Horses given 40 mg/kg body weight of green oleander leaves via nasogastric tube, consistently developed severe gastrointestinal and cardiac toxicosis.[39] Common CGs reported to be toxic are deacetyltanghinin, tanghinin, neriifolin and cerberin (2'-acetyl neriifolin).[32]

## Conclusion

The CGs from the family Apocynaceae have a potential to be developed as a new drug therapy for cardiac failure as they can inhibit $Na^+/K^+$-ATPase, as well as other steps in intracellular calcium regulation, like those involving phosphodiesterase-3 or ryanodine receptors, leading to positive inotropic effects. However, further experimental and clinical studies need to be carried out to establish the exact cardioprotective mechanisms of the drugs.

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## Conflict of interest

Authors declare no conflict of interest.

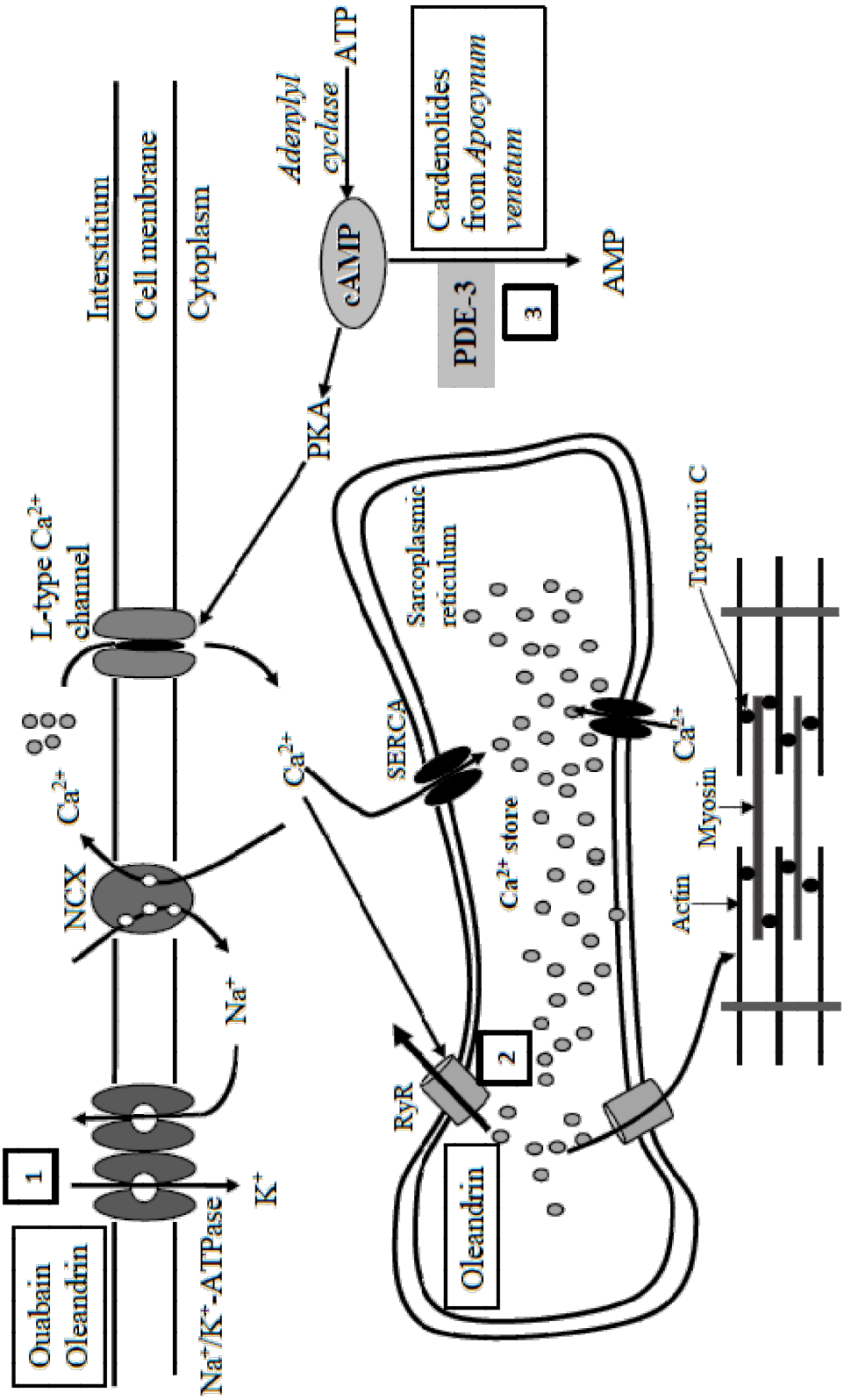


**Figure 3.** Sites of action of cardenolides from Apocynaceae plants: [1] $Na^+K^+$/ATPase (sodium pump); [2] ryanodine receptor (RyR); [3] phosphodiesterase-3 (PDE). NCX; sodium-calcium exchanger, PKA; protein kinase A; ATP, adenosine triphosphate; AMP, adenosine monophosphate; cAMP, 3',5'-cyclic adenosine monophosphate; SERCA, sarcoplasmic endoplasmic reticulum $Ca^{2+}$-ATPase.

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