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Review

A review of cardiac glycosides: Structure, toxicokinetics, clinical signs, diagnosis and antineoplastic potential

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ABSTRACT

Cardiac glycosides (CGs) are secondary compounds found in plants and amphibians and are widely distributed in nature with potential cardiovascular action. Their mechanism is based on the blockage of the heart's sodium potassium ATPase, with a positive inotropic effect. Some of the most well-known CGs are digoxin, ouabain, oleandrin, and bufalin. They have similar chemical structures: a lactone ring, steroid ring, and sugar moiety. Digoxin, ouabain, and oleandrin are classified as cardenolides, consisting of a lactone ring with five carbons, while bufalin is classified as bufodienolides, with a six-carbon ring. Small structural differences determine variations in the toxicokinetics and toxicodynamics of such substances. Most case reports of poisoning caused by CGs are associated with cardiovascular toxicity, causing a variety of arrhythmias and lesions in the heart tissue. Experimental studies also describe important similarities among different CGs, especially regarding species sensitivity. Recent studies furthermore focus on their antineoplastic potential, with controversial results. Data from research studies and case reports were reviewed to identify the main characteristics of the CGs, including toxicokinetics, toxicodynamics, clinical signs, electrocardiographic, pathological findings, antineoplastic potential and the main techniques used for diagnostic purposes.

1. Introduction

Cardiac glycosides (CGs) are widely distributed substances found in nature, produced by different species of plants and amphibians. The leading CGs in the present review are digoxin (Smith et al., 1985), ouabain (Hamlyn et al., 1991), oleandrin (Ni et al., 2002), and bufalin (Huang et al., 2015). Their chemical structure is very similar, consisting of a steroid ring, a lactone ring with five or six carbons, and a sugar moiety (Prassas and Diamandis, 2008).

Due to their chemical similarity, CGs have parallel mechanisms of action. It is believed that all are able to block the heart's sodium and potassium ATPase (NKA) current and produce a positive inotropic effect (Langford and Boor, 1996). However, small differences in their structure, especially regarding the lactone ring and sugar moiety, determine changes in toxicokinetics, clinical signs, arrhythmogenic potential and histological findings.

When examining most pharmacologic studies of CGs, there appears to be adequate data for only digoxin and digitoxin, currently commercially available for human and animal use (Doherty et al., 1975, 1978). Experimental research using ouabain is frequently associated with electrophysiological studies involving blockage of the NKA current of the cardiovascular system (Botelho et al., 2017). While oleandrin is a lesser known CG, research regarding its potential as a chemotherapeutic agent for treatment of several types of cancer has increased interest in studies of its mechanism of action and pharmacokinetics (Ni et al., 2002; Calderón-Montaño et al., 2013a; Botelho et al., 2017). Bufalin is one of the least studied CG, and the majority of researches are related to administration of crude extracts from the skins of poisonous toads commonly used in traditional Chinese medicine (Huang et al., 2015). These extracts contain multiple bufodienolides, most of which have not been chemically characterized.

Recent pharmacological uses of CGs, especially regarding anticancer therapy has sparked interest in the academic world (Mijatovic et al., 2007). However, CGs' known toxicity may restrict its clinical use. Despite recent findings on its antineoplastic potential, no consensus or standards of therapy have been established. It remains unknown the extent this treatment may achieve. We performed a review to identify the main sources of CGs, their chemical structure, toxicokinetics, clinical signs, electrocardiographic changes, pathological alterations and antineoplastic potential.

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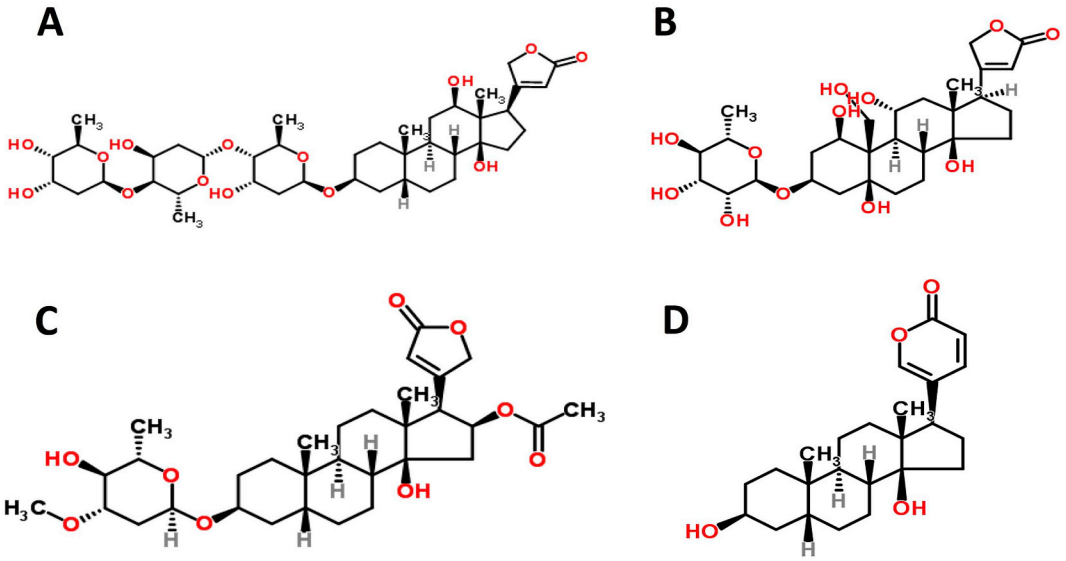

Fig. 1. Chemical structure of the main cardiac glycosides. A) Digoxin structure ($C_{41}H_{64}O_{14}$) showing the five-carbon lactone ring attached to the steroid ring (consisting of seven carbons distributed in four rings) associated with hexopyranosyl polysaccharides. B) Ouabain chemical structure ($C_{29}H_{44}O_{12}$) showing five-carbon lactone ring and mannopyranosyl monossacharide; C) Oleandrin chemical structure ($C_{32}H_{48}O_9$) showing five-carbon lactone ring, acetoxyl group, and hexopyranosyl monossacharide; D) Bufalin chemical structure ($C_{24}H_{34}O_4$) showing a steroid ring and six-carbon lactone ring.

2. Chemical structure

CGs are a large group of secondary compounds widely distributed in nature from different sources with varying applications, yet similar chemical structure. All CGs have a steroid ring attached to an unsaturated lactone ring in position 17, and most of them are also connected to a sugar moiety in position 3 (Fig. 1 and Table 1) (Schonfeld et al., 1985).

Diversity of these molecules comes from small differences in structure, especially the number of carbons from the lactone ring and associated sugar moieties. Cardenolides, mostly found in plants, have a butyrolactone ring with five carbons, while bufodienolides, mostly found in toad toxins, have a pyrone ring with six carbons (Prassas and Diamandis, 2008).

The steroid group is common to all substances, consisting of 17 carbons distributed in four rings connected to a lactone ring and different sugar residues. The most frequently found sugars are glucose, galactose, manose, rhamnose, and digitalose. CG activity is related to the lactone ring, while sugar residues are a determinant for the toxicokinetics and toxicodynamics of each substance (Brown et al., 1986; Kanji and Mac Lean, 2012). The presence of free alycones, for example, enhances absorption and metabolization of CGs, while the presence of sugar moieties, such as rhamnose, can potentiate its action by more than 25 times (Cornelius et al., 2013).

Considering this, the glycosidic form of known molecules, such as digoxin, digitoxin, and ouabain, provides better blockage of the NKA in comparison with the aglycone form. However, this phenomenon depends not only on the number and nature of the sugars, but also on the steroid structure and number of hydroxyl groups (Cornelius et al., 2013). Sugars do not have specific receptors at the interaction site, but may allow stronger binding and stability. The molecule directly attached to the steroid ring is potentially the most important to ensuring the blockage of the NKA (Cornelius et al., 2013).

3. Sources of cardiac glycosides

CGs are pharmacologically active substances produced by different species of plants and toads, that have also been identified in mammals' tissues. The most widely known are: digoxin, digitoxin, ouabain, oleandrin, bufalin, marinobufagenin, aerobufagenin, and telocinobufagin (Tables 2–4).

CGs are historically described in plants and their ingestion is associated with cardiovascular clinical signs due to the blockage of the NKA current in the heart. Ouabain is found in plants from the genera *Acokanthera* and *Strophanthus* (Cassels, 1985), digoxin in plants from the *Digitalis* genus, such as *D. lanata* and *D. purpurea* (Braga et al., 1997) and oleandrin from the plant *Nerium oleander* (Pedroza et al., 2015).

Asclepias genera, known as milkweed, is another group of plants that contains cardenolides, such as caloptrin. These CGs are often ingested by monarch butterflies (*Danaini*), which are able to absorb and store cardenolides as a result of significant adaptation of its' NKA. Such characteristic may play an important role in protection the species against their predators (Petschenka and Agrawal, 2015).

Bufodienolide poisoning also allows for the same inference of cardiac toxicity; reports of toad poisoning boosted research for the identification of different CGs and their toxins (Li et al., 2013; Huang et al., 2015). Only in the early 1990s, with the identification of endogenous digitalis-like compounds, that the search for CGs found in mammals was intensified. This made it possible to identify analogous and/or identical molecules for ouabain, digoxin, digitoxin, marinobufagenin, and telocinobufagenin in plasma samples (Hamlyn et al., 1991), urine (Goto et al., 1990), and from patients with heart failure (Gottlieb et al.,

Table 1
Chemical formula, molecular weights, and sugar residues from the main cardiac glycosides.

Cardiac glycosides	Chemical formula	Molecular weight (g/mol)	Sugar moiety
Bufalin	$C_{24}H_{34}O_4$	386.532	Absent
Digoxin	$C_{41}H_{64}O_{14}$	780.949	Hexopyranosyl polysaccharides
Oleandrin	$C_{32}H_{48}O_9$	576.727	Hexopyranosyl monosaccharide and acetoxyl
Ouabain	$C_{29}H_{44}O_{12}$	584.659	Mannopyranosyl monosaccharide

Table 2
Main families and genera of plants containing cardenolides.

Family	Main Cardioactive glycosides	Genera
Apocynaceae	Oleandrin, oleandrigenina, folineriin, odoroside, adynerin, ouabain (strophantin), cymarin, sarmentocymarin, thevetin, cerberin, peruvoside, thevetosin, neriifolin	*Adenium, Acokanthera, Strophantus, Apocynum, Cerbera, Thevetia, Nerium, Urechites, Beaumontia*
Asclepiadaceae	Periplocin, strophantidin, nigrescin, uzarin, gomphoside, calotropin, calactin, voruscharin, uscharin, 2″-oxovoruscharin, 3′-O-β-d-glucopyranosylcalactin, 12-dehydroxyghalakinoside, 6′-dehydroxyghalakinoside, ghalakinoside, calactin	*Gomphocarpus, Calotropis, Pachycarpus, Asclepsias, Xysmalobium, Cryptostegia, Menaba, Periploca, Pergularia*
Brassicaceae	Cheiroside A, cheirotoxin	*Cheiranthus*
Fabaceae	Alloglaucotoxin, corotoxin, coroglaucin, glaucorin	*Coronilla*
Hyacinthaceae	Convallatoxin	*Ornithogalum*
Leguminosae	Hyrcanoside	*Coronilla*
Malvaceae	Mansonin, cannogenol 3-O-beta-D-glucopyranosyl-(1→4)-O-beta-D-boivinopyranoside, periplogenin 3-O-beta-D-glucopyranosyl-(1→4)-O-beta-D-digitoxopyranoside and digitoxigenin 3-O-beta-D-glucopyranosyl-(1→6)-O-beta-D-glucopyranosyl-(1→4)-O-beta - D-digitoxopyranoside	*Mansonia, Corchorus*
Scrophulariaceae	Digitoxin, gitoxin, gitalin, digoxin, F-gitonin, digitonin, lanatoside A-C	*Digitalis*
Solanaceae	17-epi-11α-hydroxy-6,7- dehydrostrophanthidin-3- O-β-boivinopyranoside; 6,7-dehydrostrophanthidin3-O-β-boivinopyranoside	*Nierembergia*

Data from: Mijatovi et al., 2007; Newman et al., 2008; Roberts et al., 2015.

1992).

This discovery was followed by the correlation of endogenous CGs, blood pressure control (Hamlyn et al., 1991), and heart failure (Gottlieb et al., 1992), thus impelling new studies regarding their mechanism of action and different physiological responses.

4. Toxicokinetics

Among the substances previously cited in this study, only digoxin is approved by the Food and Drug Administration (FDA) for the treatment of heart failure and arrhythmia in human (Bocchi et al., 2009) and veterinary medicine (Atkins et al., 2009). This CG is found in the pharmaceutical market for oral and intravenous administration as an elixir, capsule, pill and ampoule. Bioavailability is close to 100% for the capsules, 70–85% for the elixir, and 75% for the pills (Lisalo, 1977 *apud* Smith, 1985). When compared to digitoxin, another CG found in therapeutic formulations, digoxin's intestinal absorption is 50% lower (Haas et al., 1972).

The half-life of digoxin can vary from species to species. In humans, the excretory half-life is between 36 and 48 h in patients with normal renal function (Smith, 1985), whereas the ouabain half-life is 11 h (Proppe, 1975), oleandrin is 2.3 h (Ni et al., 2002), and bufalin between 0.99 and 2.47 h (Cao et al., 2007; Huang et al., 2015).

Biotransformation of CGs is primarily hepatic. Digoxin can be converted into dihidrodigoxin and digitoxigenin (Doherty et al., 1978) and oleandrin into oleandrigenin (Ni et al., 2002). Tissue distribution is also very similar, as CGs have been identified in the liver, spleen, gastrointestinal tract, heart, and kidneys (Glantz et al., 1976; Ni et al., 2002; Huang et al., 2015). Oleandrin can also accumulate in the central nervous system, as it passes through the blood-brain barrier (Ni et al., 2002; Botelho et al., 2017).

Urinary excretion is considered the main elimination route for digoxin (Smith, 1985) while fecal excretion is thought to be the primary route of elimination for oleandrin (Ni et al., 2002).

Table 4
Main Cardioactive glycosides identified in species of toads.

Species	Main Bufodienolides
Amietophrynus regularis	Regularobufagin
Bufo japonicus	Gamabufagin
Bufo vulgaris	Bufotalin, bufotalinin, bufotalidin
Incilius valliceps	Vallicepobufagin
Pseudepidalea viridis	Viridibufagin
Rhinella arenarum	Aerobufagin
Rhinella marina	Marinobufagin, bufalin, aerobufagin, telocinobufagin

Data from: Mijatovi et al., 2007; Newman et al., 2008; Roberts et al., 2015.

5. Clinical signs and electrocardiography findings

Clinical and electrocardiography findings are usually associated with the accidental or experimental consumption of the CGs studied in this review, as shown in Table 5. Poisoning caused by digoxin is described as acute and chronic, according to the time of ingestion of the drug or plant. Ingestion of large amounts causes severe symptoms after six hours of exposure, while chronic consumption can cause milder symptoms, usually determined by arrhythmia and vision disturbances (Kanji e MacLean, 2012). Elderly patients with kidney failure or previously reported heart attacks are more sensitive to digoxin and may present more severe clinical signs (Bocchi et al., 2009).

Ouabain poisoning is mainly described through experimental studies. The most common clinical sign associated with ouabain is an increase in blood pressure (Davel et al., 2014). Ouabain and ouabain-like substances have been identified in human plasma (Hamlyn et al., 1991), bovine adrenocortical cells (Laredo et al., 1994), hypothalamus (Tymiak et al., 1993), and rat heart (D'Urso et al., 2004). Clinical signs and histopathological evidence associated with higher circulating levels

Table 3
Main families and genera of plants containing bufadienolides.

Family	Main Bufodienolides	Genera
Crassulaceae	Lancetoxin A and B, kalanchoside, bryotoxin A-C, bryophyllin B, cotiledoside, tyledoside A-D, F and G, orbicuside A-C	*Kalanchoe, Tylecodon, Cotyledon, Bryophyllum*
Iridaceae	Scillirosidin derivates, bovogenin A derivates	*Morae, Homeria*
Liliaceae/Hyacinthaceae	Scillarene A and B, scilliroside, scillarenia, rubelin, convalloside, bovoruboside	*Convalaria, Urginea, Bowiea*
Melianthaceae	Melianthusigenin, bersenogenin, berscillogenin, 3-epiberscillogenin	*Melianthus, Bersama*
Rannuculaceae	Helleborein, helleborin, hellebrin, helebrigenin, adonidin, adonin, cymarin, adonitoxin	*Helleborus, Adonis*
Santalaceae	Thesiuside	*Thesium*

Data from: Mijatovi et al., 2007; Newman et al., 2008; Roberts et al., 2015.

Table 5
Most common electrocardiographic findings attributed to intoxication by digoxin, ouabain, oleandrin, and bufalin.

Cardiac glycosides	Electrocardiographic findings	References
Bufalin	Sinus arrhythmia, sinus tachycardia, atrioventricular blockage, sinus bradycardia, ventricular extrasystole, and ventricular tachycardia	Kuo et al., 2007; Gadelha et al., 2014; Gowda et al., 2017
Digoxin	Bradycardia, atrioventricular blockage, sinus arrest, accelerated idioventricular rhythm, T wave inversion, ventricular tachycardia, ventricular extrasystole, supraventricular extrasystole and tachycardia, atrial fibrillation	Smith et al., 1982; Princi et al., 2000; Ramirez-Ortega et al., 2007; Joost et al., 2010; Mitchell, 2010
Oleandrin	Ventricular extrasystole, junctional rhythm, atrioventricular blockage, atrial fibrillation, ventricular tachycardia, ventricular fibrillation, sinus tachycardia, idioventricular rhythm, His blockage, sinus arrest, junctional escape rhythm, atrial flutter	Haynes et al., 1985; Le Couter and Fisher, 2002; Peymani et al., 2011; Senthilkumaran et al., 2011; Kuçukdurmaz et al., 2012;
Ouabain	Ventricular extrasystole, ventricular tachycardia, ventricular fibrillation, sinus arrhythmia, bradycardia, atrioventricular blockage	Page and Real, 1955; Germiniani et al., 1995

of ouabain are implicated in heart failure (Gottlieb et al., 1992; Naruse et al., 1994), essential hypertension (Hamlyn et al., 1991; Naruse et al., 1994), Cushing's syndrome, acromegaly, pheochromocytoma (Naruse et al., 1994), and chronic renal failure (Naruse et al., 1994; Hamlyn et al., 1996).

Oleandrin poisoning has been associated with the ingestion of *Nerium oleander*. Oleandrin is present in the whole plant, leaves, fruits, stems, roots, and flowers. In humans, poisoning is associated with criminal activity (Le Couteur and Fisher, 2002), accidental poisoning (Senthilkumaran et al., 2011), attempted suicide (Bandara et al., 2010), or by the phytotherapeutic use of the plant (Kuçudkurmaz et al., 2012). While most cases in veterinary medicine are associated with plant consumption after drought and pruning (Soto-Blanco et al., 2006; Pedroso et al., 2009).

Similar to oleandrin, bufalin poisoning is associated with contact with toad toxins from the Amphibia class, especially from the Anura order, comprising over 5000 species. Most poisonings in veterinary medicine occur when dogs mouth or bite toads stimulating a release of toxins from the parotid gland into the skin of the toads; while humans are usually exposed accidently through the ingestion of toad eggs or phytotherapeutic use of extracts or products from the toxins, such as Ch'an Su tea, used in traditional Chinese medicine (Kuo et al., 2007). Because toad toxins also contains multiple biogenic amines with potential cardio vascular effects, the interpretation of clinical signs maybe attributed to the combined effects.

6. Necropsy, histology, and electron microscopy

Necropsy exams present few macroscopic lesions, congestion and cardiopulmonary hemorrhage are common (Sonne et al., 2008; Botelho et al., 2017). Case studies in animals after ingestion of large quantities of CGs report irritation of gastric and intestinal mucosa (Papi et al., 2012; Aslani et al., 2004). Diagnosis of ingestion of plants that contain CGs is based on vegetative material in the gastrointestinal tract (Papi et al., 2012). With poisoning by bufodienolides, congestion of the oral mucosa, sialorrhea, and ulcers in the oral cavity have been reported (Sonne et al., 2008; Camplesi et al., 2013; Gadelha et al., 2014).

Histopathological exams of individuals poisoned by digoxin show myocardial necrosis, necrosis of blood vessels, focal tubular necrosis associated with proteinuria, and tubular degeneration (Bourdois et al., 1982; Ramirez-Ortega et al., 2007).

Ouabain administration can cause apoptosis of cardiomyocytes, however with no signs of necrosis, hemorrhage, or inflammation. Transmission electron microscopy analysis allows the visualization of intracytoplasmic vacuole, rearrangement of nuclear chromatin, mitochondrial damage with crest distortions, and interruption of the mitochondrial membrane (Ramirez-Ortega et al., 2007).

Reports of ingestion of *N. oleander* include myocarditis, fragmentation and degeneration of cardiomyocytes, with multifocal mononuclear infiltrate with vacuole and extensive necrosis (Pedroso et al., 2009). Kidneys may show interstitial and tubular necrosis, while the gastrointestinal tract may present congestion, enteritis, and lung focal emphysema (Aslani et al., 2007; Barbosa et al., 2008; Pedroso et al., 2009). Transmission electron microscopy reveals fragments and contracted fibers, with mitochondrial lesions (Botelho et al., 2017), as previously described in ouabain poisoning (Ramirez-Ortega et al., 2007).

The histopathology from animals poisoned by toad toxins reveals edema, hemorrhaging, and lung congestion, cardiomyocyte fragmentation and necrosis, kidney tubular degeneration and nephritis (Sonne et al., 2008; Camplesi et al., 2013; Gadelha et al., 2014).

7. Diagnostic techniques

Poisoning reports associated with cardenolide and bufodienolide ingestion are usually facilitated by a detailed history that must include a description of the CG source, e.g., plants, toad, or pharmaceuticals, the period between ingestion and hospitalization, and the progression of clinical signs. Historical evidence with clinical signs and electrocardiographic information is needed for a reliable diagnosis (Bandara et al., 2010).

The ingestion of plants containing CGs allows the species to be identified through stomach and intestinal residue (Pedroso et al., 2009). Post-mortem evaluation includes gross findings, histology, and ultrastructural lesions, as previously described. However, a reliable diagnosis is confirmed through toxicological exams.

Serum levels of digoxin and oleandrin have been successfully identified by fluorescence immunoassay and chromatographic techniques (Haynes et al., 1985; Cheung et al., 1989; Ni et al., 2002). Since there is a correlation between serum levels and the severity of poisoning, these tests not only provide diagnosis confirmation, but are also useful in determining patient prognosis.

Present studies confirm poisoning cases through necropsy tissues using High Performance Liquid Chromatography (HPLC) and Mass Spectrometry (MS) with great precision. The main samples used for CG identification are urine, serum, gastrointestinal content, kidney, brain, and heart (Galley et al., 1996; Ni et al., 2002). These analyses, however, are costly and not available in most laboratories.

8. Antineoplastic potential and future research

CGs have shown cytotoxicity towards tumor cells and research into its' antineoplastic potential has risen over the last decade (Calderón-Montaño et al., 2014; Osman et al., 2017). There are currently 1915 papers referenced in the PubMed library investigating CGs in cancer. The main hypothesis is that several cancer cells may present altered expression of NKA subunits in comparison to healthy tissues and would therefore be important targets for GCs (Mijatovic et al., 2007).

In vitro evaluations of various CGs, including digoxin, oleandrin and bufalin (Mijatociv et al., 2007; Calderón-Montaño et al., 2013a; Osman et al., 2017) against human tumor cell lines have shown cytotoxicity, mainly through formation of reactive oxygen species (Newman et al.,

2006). However, significant limitations are observed in most articles (Calderón-Montaño et al., 2014). Ideal anticancer drugs candidates should exert superior antineoplastic action than known pharmaceuticals and must cause minimal damage to healthy cells; however, very few papers use known antineoplastic drugs as a positive control.

In that regard, CGs are recognized for their cytotoxic potential as well as their very narrow therapeutic dosage, as intoxications are common. Most papers only evaluated GCs antineoplastic action against human tumor cells and determine anti-tumor activity through simple evaluation of tumor regression. This may represent a limitation and interpretations must be careful, especially because the effects on non-malignant cells are often not evaluated (Newman et al., 2006). Even low concentrations of CGs can damage both cancer and normal cells as described by (Calderón-Montaño et al., 2013a).

Recent meta-analysis showed that most pre-clinical studies uses high concentrations of CGs that may not be tolerated by humans. It also reveals that CGs are associated with higher risk of breast cancer and overall mortality rate (Osman et al., 2017).

Limit data involve anti-cancer *in vivo* therapy, usually with rodent models xenografted with human cancerous cells (Calderón-Montaño et al., 2013b; Osman et al., 2017). Several researches have indicated that most CGs exhibit 100-fold higher toxicity towards murine cells were compared to human cells, probably due to NKA subunits differences (Gupta et al., 1986). Such fact represents a limitation on data interpretation, as most CGs may selective kill human cells versus rodent cells independently from their ability to selectively kill cancer cells over normal cells (Calderón-Montaño et al., 2014).

Considering the importance of antineoplastic drug discoveries and the limitations present in CGs evaluation, future researches should aim at minimizing artifacts and evaluate both *in vitro* and *in vivo* safety regarding CGs use, especially concerning non-cancerous cells.

9. Conclusions

This paper reviewed the main characteristics of CGs, including their structure, toxicokinetics, clinical signs, diagnosis, antineoplastic potential and future use. Poisoning caused by CGs is a relatively common toxicological cause of cardiovascular poisoning cases involving animals and humans. The incorrect use of digitalis, inadvertent ingestion of plants containing cardenolides, and toad poisoning have been reported worldwide. The main clinical features are nausea, vomiting, abdominal pain, cardiac arrhythmia, and hyperkalemia. In most cases, diagnosis can be inferred by the clinical history and evolution of symptoms, but a conclusive analysis is specifically performed through an HPLC-MS of serum and necropsy tissue. Recent evaluation of antineoplastic potential of CGs should be interpreted carefully and researches must continue at pre-clinical stages to determine its effectiveness and overall safety.

Declaration of conflicting interests

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