Experimental studies on *Thevetia neriifolia* Juss—A review

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Received 18 August 1999; accepted (revised) 16 August 2000

This review article provides a comprehensive description of various studies, carried out on *Thevetia neriifolia* which is a source of several cardioactive glycosides, since early 20th century. The article provides an overview of various other medicinal herbs containing cardiac glycosides and discusses in depth various compounds isolated so far from this medicinal herb and their pharmacology. Additionally, various in vitro and biotransformation studies carried out on *Thevetia* and yield of glycosides in cultures is also detailed.

Heart failure is one of the most common causes of death and disability and is among the syndromes most commonly encountered in clinical practice. Despite important advances in the pharmacotherapy of heart failure over the past decade, the mortality rate still approaches 50%.

The cardiac glycosides have been used for centuries as therapeutic agents. Compounds containing the molecular motifs common to these agents- a steroid nucleus containing an unsaturated lactone at the C-17 position and one or more glycosidic residues at C-3 are found in many plants and several toad species, usually acting as venoms or toxins that serve as protection against predators. The steroidal cardenolides comprise one of the most interesting group of naturally occurring substances which are efficacious in the treatment of heart diseases. As components of arrow poisons, primitive people had used these substances from time immemorial.

The first comprehensive description of *Digitalis* glycosides in the treatment of congestive heart failure, as well as other ailments, are recorded in William Withering's 1785 monograph on the therapeutic efficacy and toxicities of the leaves of the common foxglove plant, *Digitalis purpurea*. Eversince, a dozen families of cardenolide- bearing plants have been recognised which include *Digitalis lanata*, *Strophanthus gratus*, *S. divaricatus*, *S. sarmentosus*, *S. thollonii*, *Nerium oleander*, *Thevetia neriifolia*, *Cerbera floribunda*, *C. dilatata*, *Beaumontia grandiflora*, *Acocanthera longiflora*, *A. oppositifolia* and *Roxpellina boivini*. The compounds are found in almost all parts of the plant, but the total amount or relative distribution in any given plant varies with ecological factors, stage of development, time of harvest and mode of drying etc.

In the 1990s, digoxin has become by far the most commonly prescribed cardiac glycoside because of its convenient pharmacokinetics, alternative routes of administration, and the widespread availability of techniques for its measurement.

A number of cardiac glycosides have been isolated from *Thevetia neriifolia*, one of which, the peruvoside is more potent than digoxin.

**Pharmacological Evaluation**

*Thevetia neriifolia* Juss was used in ancient India (1000 BC) in various skin diseases by Charak. It was classified under poisons by Sushruta in 1000 BC and was known to be a horse poison, hence named Ashwamarak or Ashwahan- the horse killer in Sanskrit. It was only in 1863 that its cardiotonic activity was discovered. Subsequently, the ouabain-like action of glycoside present in the kernels of this plant was noticed. The kernels are very bitter and when chewed produce numbness in the tongue. A decoction of the seeds acts as a violent emetic, hinders respiration and causes paralysis of the heart. However, with caution it can be used for the treatment of haemorrhoids. The seeds are also employed in criminal poisoning of the cattle. The animals fed with it, show salivation, expectoration and drowsiness. Kernels are also used as an insecticide, mashed with soap solution. Bactericidal activity has also been detected in seed oil distillates. Antifungal principle was detected in
the floral extracts against Bipolaris oryzae. Evans and Kaley reported larvicidal activity and Qamar et al. reported its utility in skin diseases. All parts of the plant produce latex, which is used for healing sores and toothache.

Every part of the plant, however, is poisonous, the kernels being the most toxic. The toxic manifestations of yellow oleander-poisoning mainly involve the cardiovascular system and the gastrointestinal tract. Jaundice and renal failure is also reported in yellow oleander poisoning. The toxic principles in yellow oleander are the cardiac glycosides; kernels contain nearly seven times as much glycosides as the leaves, stem, flowers or fruit pulp. A number of cardiac glycosides have been isolated from this laticifer, some of which are thevetin, peruvoside, and neriifolin. A number of experiments conclude ouabain or digoxin-like action on heart muscles. Positive inotropic effect followed by cardiac arrest in isolated frog, guinea pig or albino rats and emesis and muscularotropia effect was produced in cats and pigeons. Perfusion of thevetin, peruvoside or ruvoside (Thevetia glycosides) produces positive inotropic effect on hypodynamic myocardium and electrocardiographic changes. It also exhibits marked cardiotonic effect in isolated denervated heart lung preparation of dog. All cardiac glycosides, thevetin, peruvoside, neriifolin are as potent as Digitalis glycosides and appear to be promising drugs for congestive heart failure. One of these, peruvoside is even more potent than digoxin and has been marketed in Germany under the trade name Encordin.

**Thevetia Glycosides**

Thevetin was the first glycoside to be isolated from kernels. Since then as many as 15 glycosides are reported in the fresh seeds. The natural glycosides of Thevetia are triosides as they contain an aglycone unit combined with three units of sugar. The aglycone of these glycosides is digitoxigenin (or the related canthigenin and cannogenol) which is one of the aglycones of the glycosides of Digitalis purpurea. Cannogenin is the 19-oxo form and cannogenol is the 19-oxo form of digitoxigenin. The sugars are D-glucose and L-thevetose (6-deoxy-3-O-methyl-L-arabinose). An analysis of thevetoside (a mixture of cardenolides) showed presence of peruvoside, neriifolin and cerberin.

Thevetin, the first trioside to be isolated, is the major component of the seeds. It was subsequently found to be a mixture of two triosides, cerberoside (thevetin-B) and thevetin A. A small proportion of another trioside, 2'-O-acetyl cerberoside is also associated with thevetin. The separation of thevetin into pure components, thevetin A and cerberoside was achieved by partial chromatographic and counter current techniques by several workers. By the counter current technique, thevetin-A and cerberoside were obtained in a ratio of 1:2. 5-Methyl ether of apigenin is also reported from seeds. The monosides separated from the seeds include neriifolin, cerberin (2'-O-acetyl neriifolin), peruvoside, thevenerin (ruvoside) and peruvosidic acid (perusitin). These glycosides do not occur as such in the seeds and are formed as a result of enzymic hydrolysis of the triosides. Peruvoside is obtained by partial hydrolysis of thevetin A; upon reduction, peruvoside yields thevenerin (ruvoside). Peruvoside and thevenerin are related as aldehyde and alcohol to each other. Peruvosidic acid is obtained upon oxidation of peruvoside by CrO₃.

During the isolation of cerberoside (thevetin-B), part of the trioside is partially hydrolyzed, losing two molecules of glucose, yielding a mixture of cerberoside and neriifolin. The enzyme thevetinase is responsible for the hydrolysis. If incipient enzymic action is carefully prevented, as high as 4.5% trioside is obtained, with correspondingly insignificant amount of monoside. In contrast, if the enzymic action is deliberately promoted such as by fermenting the seeds, almost the entire quantity of the trioside disappears, concomitantly monoside becomes high, and isomeric neriifolin yields cerberin and isomeric neriifolin-4'-monoacetate. Partial acetylation of neriifolin yields cerberin and isomeric neriifolin-4'-monoacetate.

Thevetia glycosides are chemically closely related to each other as represented in Chart 1.

The aglycones as well as the glycosides undergo isomeric changes in the presence of bases.

A number of known and new glycosides have also been isolated from various parts of this plant, albeit in small amounts. Abe et al. isolated several new cardiac glycosides from air dried leaves including neriifolin (C₉H₁₄O₃), solanoside, thevetoside (A-G). A new cardenoline, neriifoside 3β-O-α-L-arabinoside was isolated from fresh uncrushed leaves along with peruvoside. Besides, a steroid, 4,16-pregnan-12β-
Thevetin (Crude)

Cerberoside
(ThevetinB)

enzyme
action
-2 mols
glucose

enzyme
action
-2 mols
glucose

Special
reduction process

Nerifolin

Peruvoside

Peruvic
acid

Reduction

acetylation

Cerberin
(2'-O-acetylnerifolin)

4'-O-acetylnerifolin

Theverin (ruvoside)

Source: Wealth of India

Chart 1

hydroxy-3,20-dione and four pentacyclic triterpenes: oleanolic acid, ursolic acid, α-amyrin acetate, hitherto
unreported from this source, have been isolated.

Amongst irridoids, theviridoside was the first to be
isolated and characterized in T. neriifolia. Iridoids are
more stable in flowers and fruits as compared to leaves.
Theveside is completely destructed in yellow senescent
leaves due to blocked chromogenic groups. Two
minor irridoids, 10-O-β-D-fructofuranosyl thevirido-
side and 6'-O-β-D-glucopyranosyl theviridoside have
been reported recently from roots.

Leaves of T. neriifolia also contain flavonol sinapoyl
glycosides. Abe et al reported few of these, kaempferol 3-glucosyl (1-4) [6'-sinapoyl glucosyl] (1-2)
galactoside and 3-[2'-sinapoyl glucosyl] (1-4) [6'
inapoyl glucosyl] (1-2) galactoside, and quercetin 3-
[6'-sinapoyl glucosyl] (1-2) galactoside along with
known compounds kaempferol and quercetin 3-
glucosyl (1-2) galactoside. Dinormonoterpenoids
and their apiosyl glycosides have recently been reported.
All parts of the plant except the flowers are known to
contain cardiac glycosides. However, Gunasegaran and
Nair reported flavol glycoside, thevetofolin along with
3-galactosides of quercetin and tamarixetin and 3-
digalactoside of quercetin extracted by exhaustive
extraction of fresh flowers with 90% ethanol. Later
they also reported two phenyl propanoids from
flowers. Apigenin-5-methyl ether occurs in seed
shells of T. neriifolia.

Detection
For detection and quantification of Thevetia cardiac
glycosides various techniques have been used, which
include radioimmunooassay, HPLC determination,
enzyme linked immunosorbent assay and fluorescence polarization.

In vitro Studies
Owing to short life of seeds, dormancy, poor
germination and difficulty in propagation through
cuttings, alternative methods of multiplication become
imperative. Besides, poor stability of cardenolides
towards acids and bases, the presence of rare sugar
residues hinders their chemical synthesis and restrict
their extraction and isolation from natural sources.
Since glycosides are prone to hydrolytic enzymes, they
are found in very low amounts in plants. Hence,
pharmaceutical industry looks forward to undertake a
biotechnological approach for the synthesis of such
important cardenolides. In vitro raised cultures would
prove profitable, as true to type individuals with
 genetic homogeneity of desirable characters can be mantained in the resultant plantlets. Also, predictable yield of glycosides can be ensured. A number of cardenolides have been reported in in vitro cultures of

\[ \text{Digitales}^{61-64} \]

**In vitro** regeneration of *T. nerifolia* has been attempted by a few workers. Initially, regeneration and rooting from stem cuttings was attempted with several growth regulators. Kumar and Sharma\(^{65}\) reported positive role of IAA in the induction of roots and regeneration of shoots, while it was adversely affected by GA₃. Lower concentrations of coumarin promoted root formation and inhibited shoot regeneration, whereas higher concentration retarded both. In vitro proliferation and good growth with rooting could be achieved with NAA and kinetin\(^{66}\) in cotyledonary callus raised on WB medium with NAA + kinetin\(^{67,68}\). They could detect thevetin in callus cultures by TLC. However, the percentage of thevetin decreased with age of callus and disappeared completely after six months. Yield of peruvoside and nerifolin could be successfully modulated using various stress causing agents under in vitro conditions\(^{69}\). The yield obtained was however explant dependent.

Modulation of cardenolide biosynthesis failed in such cultures when tried using different sugars (4% sucrose, glucose and maltose), yeast extract, casein hydrolysate, malt extract, coconut milk, amino acids or growth hormones with Wood and Brown (WB) medium. Callus tissues grown for 1 month under different light conditions and with precursors (cholesterol + progesterone) also failed to produce thevetin. Thevetin-A and thevetin-B were detected in cultures raised on Murashige and Skoog's medium\(^{70}\).

Effect of exogenous precursors on in vitro secondary metabolism was also studied in *T. nerifolia*. Transformation of cholesterol to pregnenolone and progesterone was observed in suspension cultures within 24 hr. Increase in progesterone concurred with decrease of cholesterol and pregnenolone and vice-versa\(^{70}\). Later Dantas-Barros \(^{71}\) reported cardenolide formation in cell suspension cultures. In vitro cultures opened up the possibility of using plant cells for the transformation of steroids as is commonly done with microorganisms. Results of biotransformation with plant cells, of different substances, in particular of steroids are compiled in several reviews\(^{72-75}\). A comparison of biotransformation potential of *Thevetia nerifolia* and *Digitalis lanata* cell cultures revealed that cardenolides common to the plant species were metabolized by plant cell cultures more rapidly than cardenolides uncommon to that species. Both glucosylation and deacetylation could be observed *Thevetia* and *Digitalis* cell cultures, while deglucosylation and oxidation only occurred with the *Thevetia* and 12β-hydroxylation only with *Digitalis* cell cultures. Furthermore, the *Thevetia* cultures were not capable of transforming methyl digoxin into methyl digoxin\(^{76,77}\).

Barros \(^{78}\) reported biotransformation of the synthetic substrate, ethyl 2-acetyl amino 2-carbethoxy-4-(phenyl sulphonyl)butanoate by cell suspension cultures of *Catharanthus roseus* and *Thevetia nerifolia*. It was found that only three of the six cell lines of *T. nerifolia* tested, biotransformed the 2-test substrate into a new product, ethyl 2-acetyl amino-2-carbethoxy-4-(phenylsulphonyl)-butanoate, through a selective oxidizing process not previously described. Cardenolide content also varies in different cell lines\(^ {79}\). Such a biotechnological process could be of great interest for the production of new chemical compound involving stereospecific chemical reactions hitherto unreported through chemical synthesis.

In view of the ever increasing demand for novel and more potent cardenolides, additional efforts need to be made to ensure constant and stable supply of these drugs. Owing to the presence of highly stereospecific sugar moieties, chemical synthesis is hindered hence, attempts should be made to explore the possibility of detecting cardenolides in in vitro cultures.

**Acknowledgements**

AG is grateful to CSIR for a fellowship. Thanks are also due to all the colleagues in PTC Lab, Jamia Hamdard for help in various ways.

**References**

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