CARDIOVASCULAR EFFECTS OF A WITHANOLIDE FROM *WITHANIA COAGULANS*, DUNAL FRUITS*

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Summary : A new withanolide, with a unique chemical structure similar to the aglycones of the cardiac glycosides, with mol. wt. 488.6, m. p. 260–261°, isolated from the fruits of *Withania coagulans*, was screened for cardiovascular effects. At doses of 5 *mg/kg* body weight, the withanolide produced a moderate fall of blood pressure in dogs (34 ± 2.1, *mm Hg*) which was blocked by atropine and not by mepyramine or propranolol. In rabbit Langendorff preparation and ECG studies, it produced myocardial depressant effects but in perfused frog heart it produced mild positive inotropic and chronotropic effects.

Key words : *Withania coagulans* Withanolide cardiovascular effects

INTRODUCTION

A new group of steroidal lactones called withanolides has been recently isolated from different species of Solanaceae family mainly *Withania somnifera* (1, 10,11, 15, 21). Some of the withanolides mainly Withaferin A isolated from leaves of *Withania Somnifera* have been reported to possess remarkable antibacterial, (3,12), antitumor (17,18), antiinflammatory and antiarthritic properties (2,16). In fact the isolation of an antibacterial and antitumor agent viz withaferin A has created a great interest among scientists of different disciplines and intensive research on *Withania somnifera* and other species of the same family is being pursued by different workers with a view to isolate new and potent drugs from among these plants.

During our studies on *Withania coagulans* fruits, one such withanolide with a molecular weight of 488.6, m.p. 260-261°C was isolated from aqueous extract of the

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fruits and identified 3 β-hydroxy-2,3-dihydro-withanolide F. It is a new compound of unique chemical structure and of high biogenetic interest (22). The withanolides have close structural similarity to aglycones of the cardiac glycosides in possessing a 6-membered unsaturated lactone attached to a steroidal ring at C_{20} instead of C_{17} (Fig. 1). As the pharmacological activity of the glycosides resides in aglycones (6, 20), the withanolide isolated from *Withania coagulans* fruits was screened to find out whether, like the aglycones, this compound has any effect on the cardiovascular system.

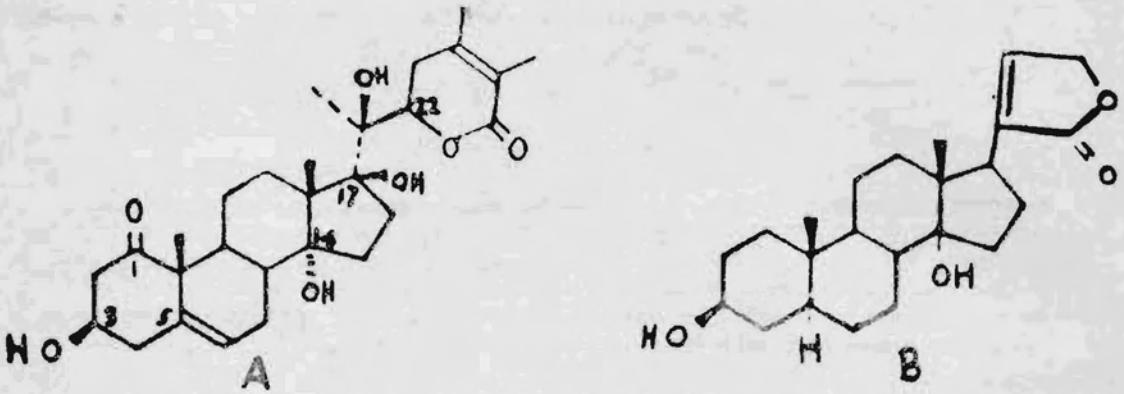

Fig. 1 : Structural resemblance of Withanolide to digitoxigenin.
A = Withanolide, B = digitoxigenin.

MATERIAL AND METHODS

The withanolide was isolated from fruits of *Withania Coagulans* (22). A 1.0% solution was made in absolute alcohol and diluted with saline before use for dog experiments and rat hind limb preparation. For other experiments 1% suspension of the withanolide in 1% sodium carboxymethyl cellulose solution was used. Unless otherwise stated, the drug was administered through the femoral vein. Equivalent quantities of the solvents were also administered by the same route. The following studies were carried out :

Blood pressure and respiration : Mongrel dogs (12-15 *kg*) anaesthetized with 30 *mg/kg* pentobarbitone sodium were used for recording blood pressure and respiration. Blood pressure was recorded from a common carotid artery by a mercury manometer and respiration from trachea by means of Brodie's tambour. Carotid baroreceptor reflex was tested by occluding both the common carotid arteries for 30 sec.

Perfused frog heart was set up as described by Burn (4).

Perfused hind limb preparation of albino rats was set up as described by Burn (4).

Rabbit isolated heart preparation was set up by Langendorff technique (13).

E.C.G. studies were conducted on dogs anaesthetized with pentobarbitone sodium (30 *mg/kg*, iv). Records were made on a single channel ink recording electrocardiograph.

RESULTS

The withanolide in alcoholic solution at a dose of 5 *mg/kg* produced an immediate sharp fall followed by moderate fall of blood pressure (34±2.1 mmHg) in dogs (n=5). The immediate sharp fall was found to be due to alcohol. The hypotensive response was blocked by atropine (2 *mg/kg*) but not by propranolol (1 *mg/kg*, Fig. 2). It was not blocked by mepyramine (2 *mg/kg*) also. The effects of acetylcholine, adrenaline and nicotine at the usual doses or carotid occlusion (30 sec.) were not changed after the administration of withanolide. The hypotensive response was less with suspension of the withanolide at the same doses.

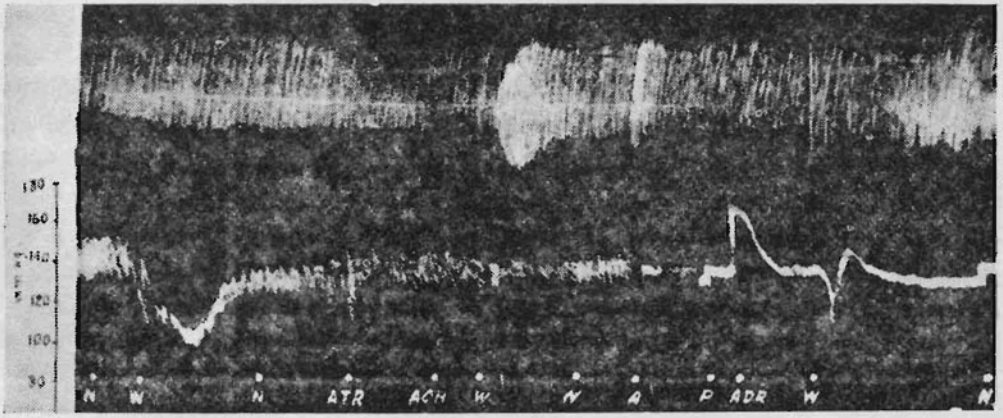

Fig. 2 : Effect of withanolide on dog blood pressure and respiration. Upper tracing respiration and lower blood pressure.

N= Normal ; W=Withanolide, 5 *mg/kg*; ATR=atropine, 2 *mg/kg*; ACH=Acetylcholine, 1 µg/kg ; A=alcohol equal to solvent of withanolide ; P=propranolol, 1 *mg/kg* ; ADR= adrenaline, 2 µg/kg.

In perfused frog heart (n=10), 2 *mg* of the withanolide in the suspension produced positive inotropic and chronotropic effects which were blocked by propranolol. The heart rate increased from 61.2±1.39/min to 77±1.94/min which was significant ($P<0.01$) whereas the amplitude of contractions increased from 34.0±3.5 *mm* to 40.1±3.2 *mm* ($P<0.01$).

In rat hind limb preparation, withanolide (1 *mg)* caused insignificant ($P>0.05$) vasoconstriction in all the 5 animals. The volumes of outflow of perfusion fluid with solvent and drug were 5.2±0.20 *ml*/4 min and 4.66±0.19 *ml*/4 min respectively.

In rabbit Langendorff preparations (n=5) 2 *mg* of withanolide produced negative inotropic and chronotropic effects. The heart rate decreased from 71±2.4/min to 19±0.28/min and amplitude of contractions decreased from 14.6±1.1. *mm* to 6.0±0.8 *mm* ($P<0.01$). However, there was no significant alteration in coronary flow which was 6.36±0.25 *ml/min* and 6.12±0.24 *ml/min* with solvent and withanolide respectively.

A dose of 5 *mg/kg* given in the form of infusion in saline over 15 min did not produce any alteration in ECG. However, the bolus dose of 10 *mg/kg* in alcohol caused depression of S-T segment (Fig. 3).

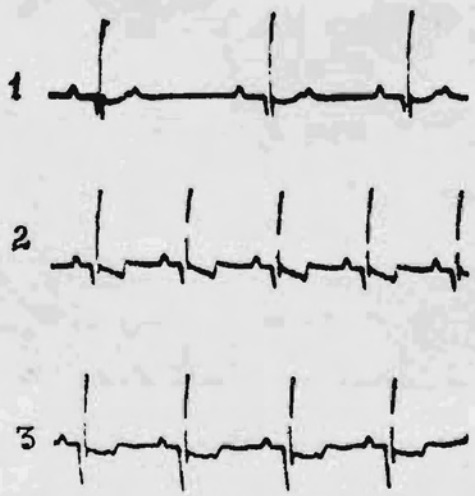

Fig. 3 : E.C.G. changes in dog s heart as recorded from lead II at a bolus dose of 10 *mg/kg*, iv of the withanolide. 1. Normal, 2. Immediately after ; 3. 15 min after the withanolide.

The withanolide at 5 *mg/kg* dose increased the rate and depth of respiration. The respiratory rate increased from 18±1.4/min to 65±5.3/min in dogs (n=5) which was significant ($P<0.01$), (Fig. 2).

DISCUSSION

In the present investigation, the withanolide caused 34±2.1 mm Hg. fall in blood pressure when administered in alcoholic solution at 5 *mg/kg* dose. The hypotensive effect was blocked by atropine (2 *mg/kg*) but not by propranolol (1 *mg/kg*) or mepyramine (2 *mg/kg*). From the results it is evident that the hypotensive effect is cholinergic in nature and the possible site of action may be the myocardium. The ECG studies on dog and the negative inotropic and chronotropic response on the rabbit Langendorff preparation further support this contention.

Positive inotropic and chronotropic effects observed in perfused frog heart could be due to species differences as many other lactones and glycosides exert different effects in amphibians and mammals (6,14,20).

Although the effects of the withanolide are not similar to those of cardiac glycosides, the possibility of cardiotonic response with other withanolides cannot be ruled out at present since screening of a large number of cardiac glycosides and aglycones has revealed that the position of lactone ring attached to ring D, other groups present in the steroidal ring and spatial arrangement in the steroidal ring system of the cardiac glycosides influence significantly their cardiotonic activity (5-9,19,20).

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