Cardiac and Extracardiac Activity of an Ethanolic Extract of Leaves of *Isoplexis canariensis*

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Abstract: The paper deals with the effects of a glycosidal extract of *Isoplexis canariensis* (Schrophulariaceae), grown in the Canary Islands, on isolated auricle of rabbits, isolated ileum of guinea-pigs, isolated jejunum of rabbits, urinary excretion of rats and $^{86}Rb^+$ uptake by red cells. The extract produced an increase in the contraction force of auricles, contractile response in guinea-pigs ileum, hypermotility followed by paralysis in the rabbit jejunum, diminution of the urinary excretion in rats and decrease of the $^{86}Rb^+$ uptake by red cells. Except for the antidiuretic action, the pharmacological activity of the principles contained in the extract is similar to that of the digitalis compounds.

Introduction

The presence of steroid glycosides in leaves of *Isoplexis canariensis* (Schrophulariaceae), grown in the Canary Islands, was described by González and Calero (1, 2). Five compounds have been isolated: canarigenin-glucoside-digitoxoside, canarigenin-glucoside-canaroside, xysmalogenin-glucoside-canaroside, uzarigenin-glucoside-canaroside and canarigenin-glucoside-fucoside (1, 2, 3, 4). Although cardiotonic activity of these compounds has been found previously (6, 5) it was decided to broaden the information about their pharmacological activity. The present paper deals with the effect of an ethanolic extract of leaves of the plant on the spontaneously beating rabbit auricle, the isolated guinea-pig ileum, the isolated rabbit jejunum, the rat urinary excretion and the $^{86}Rb^+$ uptake by red cells.

Material and Methods

Preparation of the extract

The plant was collected in June at Las Mercedes Mount (Tenerife, Canary Islands). 500 g of the dried leaves were extracted with ethyl alcohol for five days. After filtration, the alcohol extract was evaporated to dryness under reduced temperature. The viscous residue was repeatedly washed with sulphuric ether. After removal of chlorophyl, the extract was dissolved in methyl alcohol, and tanins were precipited by adding Pb $(OH)_2$. The filtrate was extracted with ethyl acetate and concentrated to a small volume in vacuo. The concentrated solution formed crystals after evaporation at room temperature. After recrystallization in methanol, feather like needles were obtained. The extract gave positive reactions against Fehling, Legal, Keller-Killiani and Kedde reagents. The active principles were detected by thin layer chromatography on silica gel. The UV and IR spectra were narrowly similar to those of digoxin. The extract was soluble in methanol, acetone and ethyl acetate, moderately soluble in ethanol, poorly soluble in water and insoluble in sulphuric ether, petroleum ether and benzene. For pharmacological experiments, suitable concentrations of about 25 mg ml^{-1} were prepared in propylene glycol-ethanol-water (40/10/50).

Pharmacological methods

Isolated auricle of rabbit

Male rabbits weighing 1100–1500 g were killed by a blow on the head. The chest was opened, the heart removed as quickly as possible and placed in Krebs-Ringer solution. The auricles were dissected free from all other tissues and threads were tied to the tips of each auricle. The preparations were mounted in Krebs-Ringer solution at 31° C through which a stream of 5 % CO_2 – 95 % O_2 mixture was continuously bubbled. Initial tension of 1 g was applied, and after a period of 30 min of stabilisation the spontaneous isometric contractions were recorded in a Beckman R-511 dynograph through a Statham force-displacement transducer. The control group comprised twelve auricles whose contractions were recorded for 60 min in the presence of the vehicle used for dissolving the extract. The effect of the extract was studied for 60 min at the following doses: 60, 120 and 240 mcg ml^{-1}. Parallel experiments were carried out with digoxin 2 ng ml^{-1}. Each dose was tested on twelve auricles.

Isolated guinea-pig ileum

The animals were killed as for the rabbits. The abdomen was opened, a length of ileum removed and placed in Tyrode's solution. Pieces of ileum were dissected free from surrounding tissues and mounted in Tyrode's solution at 37° C through which a mixture of 5 % CO_2 and 95 % O_2 bubbled continuosly. The isotonic contractions of the preparation were recorded on a kymograph through a frontal writing lever. The load applied to the lever was 0.5 g. After stabilisation, a cumulative dose: effect assay was verified. Ten pieces of ileum were used.

Isolated rabbit jejunum

Pieces of jejunum were prepared in a way similar to that described for the isolated ileum of guinea-pig. Spontaneous motility of jejunum was recorded on a Harvard dynograph through an isotonic muscle transducer. A non cumulative assay was performed on ten preparations.

Urinary excretion in rats

The procedure reported by Colot (7) was employed. The animals were starved during a period of 18 h prior to the experiments. Following this period, the animals received a solution of 0.9 % NaCl (50 ml kg^{-1}) by intragastric route, followed by the administration of the extract, 10 and 20 mg kg^{-1}, by intraperitoneal route. The control group received only the load of 0.9 % NaCl and a volume of the vehicle similar to that used for dissolving the extract. The animals were then individually placed in metabolism cages. The urine was collected during six hours, and the urinary volumetric excretion was calculated (percent of the total volume administered). The concentration of Na^+, K^+, and Cl^- was measured on samples from the total urine of six hours.

$^{86}Rb^+$ uptake by red cells

The procedure described by Bernstein and Israel (8) was used. Red cells were separated from heparinised blood by centrifugation and washed twice with potassium-free Krebs-Henseleit solution at 4° C. 0.5 ml of red cells was mixed with potassium-free Krebs-Henseleit solution containing $^{86}Rb^+$ (final concentration 5 mM). After 40 min of incubation at 37° C, the suspension was centrifugued and the erythrocytes washed with choline chloride (150 mM) and ouabain (0.3 mM). An ali-

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quot was removed for counting total radioactivity. The effect of the extract was studied at concentrations of 100, 200, 400, 800, 1600 and 3200 ng ml^{-1}.

Statistical calculations
Student's t test was used for comparison between means.

Results

Isolated auricle of rabbit
The inotropic and chronotropic effects of the extract are depicted in Fig. 1 and 2, respectively. As compared with controls, the extract produced a steady increase in the contraction force, in such a way that 50 min after its administration the peak-effect had not yet been reached. The response exhibited a clear dose : effect relationship. Concerning the chronotropism, the two lower doses did not cause significant changes. However the highest one induced a slight increase in the frequency from the 30th min onwards. As expected, digoxin gave rise to an increase of the inotropism without changes in the chronotropism.

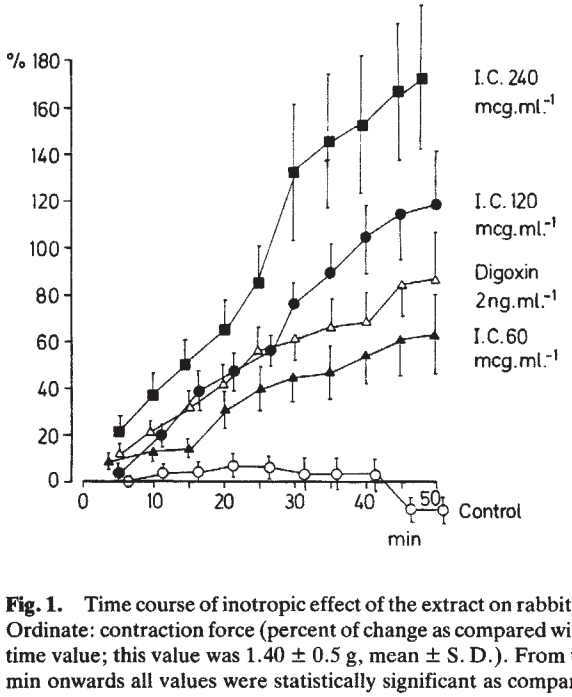

Fig. 1. Time course of inotropic effect of the extract on rabbit auricle. Ordinate: contraction force (percent of change as compared with the O time value; this value was 1.40 ± 0.5 g, mean ± S. D.). From the 15th min onwards all values were statistically significant as compared with controls.

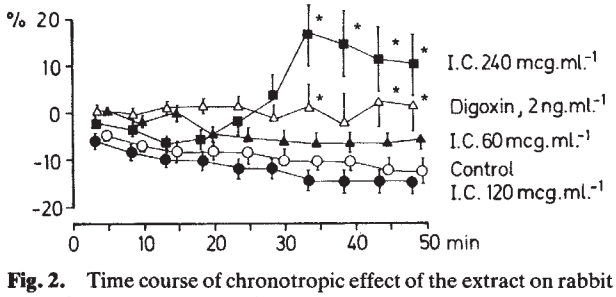

Fig. 2. Time course of chronotropic effect of the extract on rabbit auricle. Ordinate: beats/min (percent of change as compared with the O time value; this value was 106.4 ± 10.0 beats/min, mean ± S. D.) *) $p < 0.05$.

Isolated guinea-pig ileum
The extract produced contractile responses which were related in a dose dependent manner (Fig. 3).

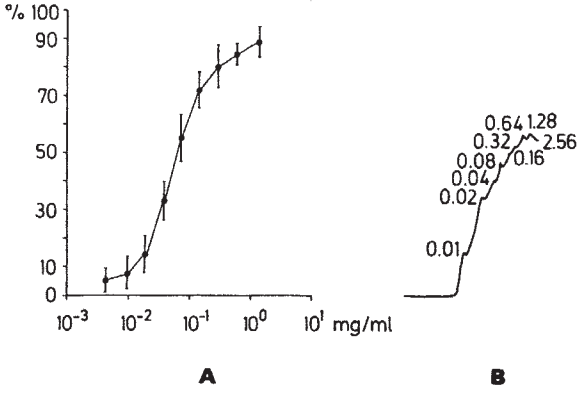

Fig. 3. Contractile response of guinea-pig ileum. A) Dose : effect relationship. B) Graphical recording of one of the experiments (numerical symbols represent mg ml^{-1} of the extract).

Isolated rabbit jejunum
The extract caused a biphasic effect, a short period of hypermotility being followed by paralysis. After washing, the normal motility was recovered. Despite the consistence of these effects, a clear dose : effect relationship could not be established. For that reason, presentation of quantitative data has been omitted.

Urinary excretion in rats
The extract produced a decrease in the urinary volumetric excretion accompanied by an increase in the urinary excretion of both K^+ and Cl^- and a decrease in the excretion of Na^+. See Figure 4.

$^{86}Rb^+$ uptake by red cells.
The extract produced a marked inhibition of the Rb uptake (Table I). A maximal decrease of about 70 % was obtained with the highest doses.

Table I. Effect of the Extract of *Isoplexis canariensis* on the $^{86}Rb^+$ uptake by Red Cells

	mM of $^{86}Rb^+$ taken up/mcl/min
CONTROL	25.8 ± 4.2
I.C. 100 ng. ml^{-1}	12.7 ± 3.9*
I.C. 200 ng. ml^{-1}	10.3 ± 4.0*
I.C. 400 ng. ml^{-1}	9.1 ± 3.5*
I.C. 800 ng. ml^{-1}	8.2 ± 3.6*
I.C. 1600 ng. ml^{-1}	8.0 ± 3.6*
I.C. 3200 ng. ml^{-1}	8.1 ± 3.5*

I.C. = Extract of *Isoplexis canariensis*. *) $p < 0.05$, as compared with control. Values of $^{86}Rb^+$ uptake represent mean ± S.D. of six experiments.

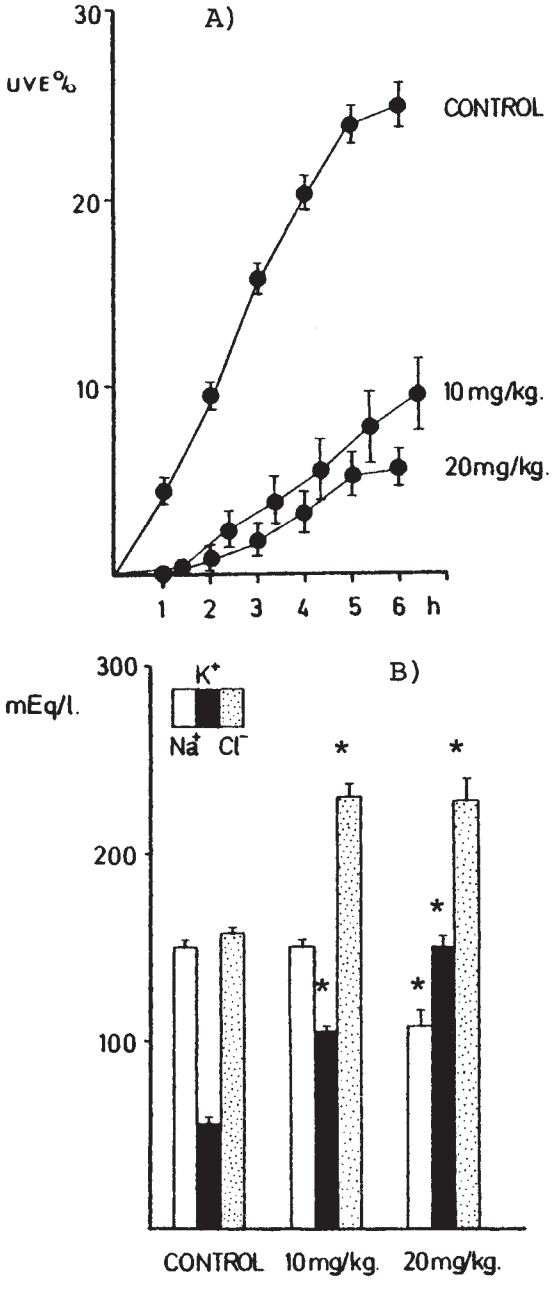

Fig. 4. A) Time course of the effect of the extract on urinary volumetric excretion (UVE). As compared with controls, all values were statistically significant. B) Action of the extract on urinary excretion of ions. *) $p < 0.05$, as compared with control values.

Discussion

As it has been shown, the compounds contained in the extract gave rise to an increase of the contraction force of the rabbit atrium, which was accompanied by only slight changes in chronotropism. In this respect, the increase in the rate observed by the 30th minute onwards with the highest dose, might well be the earliest sign of the extract toxicity. Both the magnitude of the extract inotropic effect and its similarity with the time course of the digoxin effect, make these findings interesting enough to perform future studies with each constituent separately.

Cardioactive glycosides are known to increase the tone of the intestinal smooth muscle as well as abolishing peristaltic movements (9, 10,11). In the present study the extract first contracted and then paralysed the rabbit jejunum. On the other hand, the guinea-pig ileum, a preparation lacking peristaltic activity, was only contracted by the extract. Therefore, the effect of these compounds on the intestinal muscle appears to be clearly similar to that produced by Digitalis compounds.

Unexpectedly the extract produced an antidiuretic effect. There is no satisfactory explanation for this finding, which clearly contrasts with the slight diuretic effect previously observed in this laboratory (6) with a similar extract. On that occasion the doses used were bigger (ten fold, approximately) in such a way that an osmotic diuresis, depending on vegetal saccharide overload, was suspected. In fact, the presence of sugars was noticed in the urine. Concerning the present results, it is evident that the action of a mixture of products on urinary excretion is unpredictable and better information will be obtained by studying each compound separately. For instance, the authors have observed that uzarigenin-glucoside-canaroside, one of the glycosides presumably present in the extract, produced a diuretic effect in a dose related manner (unpublished data)

In spite of the various mechanisms proposed to explain the inotropic effect of the cardiac glycosides (12), an inhibitory action on $Na^+ : K^+$-ATP-ase is commonly accepted. A feasible method to study such an inhibition has been described by Bernstein and Israel (8) in which the amount of $^{86}Rb^+$ taken up by washed red cells in the presence of ouabain is measured. The results here obtained demonstrate that the extract, used instead of ouabain, caused a marked inhibition of the $^{86}Rb^+$ uptake, as expected.

Finally, it must be pointed out that oral infusion of dried leaves of Isoplexis canariensis is used in the Canary Islands as a remedy against diabetes mellitus. This action has not been confirmed in animal experiments (6). However, even though the plant does have some hypoglycaemic effect in humans, it is clear that diabetic patients must not be unnecessarily exposed to the cardiac effects of the glycosides here studied.

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