

PHARMACOLOGY AND TOXICOLOGY OF CHACONINE AND TOMATINE

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ABSTRACT

The pharmacological responses produced by α chaconine and tomatine on guinea pig ileum, on the isolated electrically stimulated frog ventricle, and recordings of EEG, ECG, respiration and blood pressure in the rabbit showed no essential differences from those produced by α solanine. The LD₅₀ values of chaconine and solanine in the mouse and rabbit are also similar and suggest that compounds other than these are probably responsible for the predominant toxic effects of certain hybrid potatoes in man and animals. The failure of the three glycoalkaloids to produce a significant teratological effect in the chick embryo lends no support to the hypothesis that they may be the teratogens responsible for certain congenital malformations in man.

INTRODUCTION

Nishie and coworkers (1971) determined the pharmacological properties of the glycoalkaloid, solanine, in an attempt to evaluate its possible rôle in the potato poisoning of humans and animals. They found, in rats that solanine was poorly absorbed by the oral route and cleared very rapidly from the blood and GI tract by urinary and fecal excretion. These findings suggested that other glycoalkaloids or phytoalexins in the potato might make a relatively greater contribution than solanine to the overall toxicity of certain varieties of hybrid potatoes.

Renwick (1972) hypothesized that the severity of late blight (*Phytophthora infestans*) in potatoes might be related to the incidence of spina bifida cystica and anencephaly in man. He also postulated that solanine might be one of the teratogens responsible for these congenital malformations.

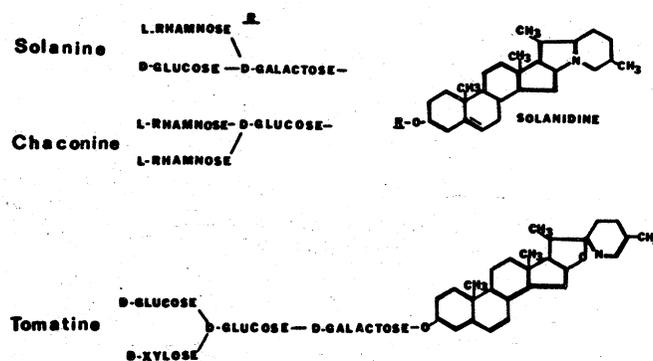
We have undertaken to determine which of the several glycoalkaloids present in the potato might exert the predominant toxic effect, and to evaluate their potential teratogenicity. The pharmacology of chaconine and tomatine has been studied and compared with that of solanine.

MATERIALS AND METHODS

The tomatine and α solanine (Fig. 1) used in these studies were purchased from Aldrich Chemical-Co., Inc., Milwaukee, Wisconsin. α Chaconine was prepared from leaves of *Solanum chacoense* by E. A. Talley using the method of R. Kuhn and I. Löw (1961), followed by chromatographic separation from other glycoalkaloids on columns of potato starch-silica gel. The elution solvent was the lower layer from a mixture of chloroform,

ethanol and 1% aqueous ammonia in the volume ratio of 3:3:1 (H. Roensch and K. Schreiber, 1966). The crystalline material so obtained (7.8 g), was recrystallized from 80% aqueous ethanol: yield 6.7 g. Specific rotation, $[\alpha]_D^{27} -84.01^\circ$ in pyridine (Lit. -85° , Kuhn and Löw, 1954). Melting point (Fisher-Johns), $240-242^\circ$ (softens 235°C) (Lit. 243° , *ibid.*). Acid hydrolysis produced only rhamnose, glucose and solanidine, as shown by thin layer chromatography. Purity of the final product was also checked by gas chromatography, mass spectrometry and thin layer chromatography.

Fig. 1



Male Swiss-Webster mice weighing 18-22 grams were used in the determination of the intraperitoneal median lethal dose (LD_{50}) by the log probit method (Miller and Tainter, 1944). The parasympathetic effects of the glycoalkaloids were determined on guinea pig ileum in Krebs bicarbonate Ringers solution (pH 7.2) at 38°C . The positive inotropic effects of the compounds were studied at ambient temperature on the electrically stimulated isolated frog ventricle preparation (Hajdu and Szent-Gyorgy, 1952). The electroencephalogram (EEG), electrocardiogram (ECG) and

respiration were recorded with a Beckman Type R Dynograph¹ in unanesthetized white New Zealand rabbits weighing 3.5-4.0 kilograms, by use of the intraperitoneal (i.p.) route of administration of test compounds. The contractions of guinea pig ileum and isolated frog ventricle were measured with a Statham force-displacement transducer, and the EEG signals were recorded from implanted skull electrodes in the rabbit (Nishie et al., 1969). Test compounds were dissolved in either propylene glycol or an aqueous 0.1 N HCl solution.

The possible teratogenic effects of the compounds were tested after injection of the glycoalkaloids dissolved in a mixture of propylene glycol and 0.1 N HCl (0.05 ml/60 gram egg) into the yolk sac of the fertile eggs prior to incubation and, in a separate experiment, on the 96th hour of incubation. The eggs were candled on the 7th and 14th days of incubation and dead embryos were removed and examined. The hatched chicks were weighed and examined for malformations. The χ^2 test was used to evaluate the differences in the incidence of malformations and in the hatchability between treated and control groups.

RESULTS

Acute Toxic Effects of Solanine, Chaconine, and Tomatine in Mice and Rabbits

The LD₅₀ values (Table 1) did not differ significantly among the three compounds.

¹Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.

Table 1. Lethal Doses in Mice (ip)

Glycoalkaloids	LD ₅₀ ± S.E. μM/kg(mg)
α Chaconine	32.3 ± 2.05(27.5)
α Solanine	34.5 ± 2.3 (30.0)
Tomatine	32.4 ± 1.25(33.5)

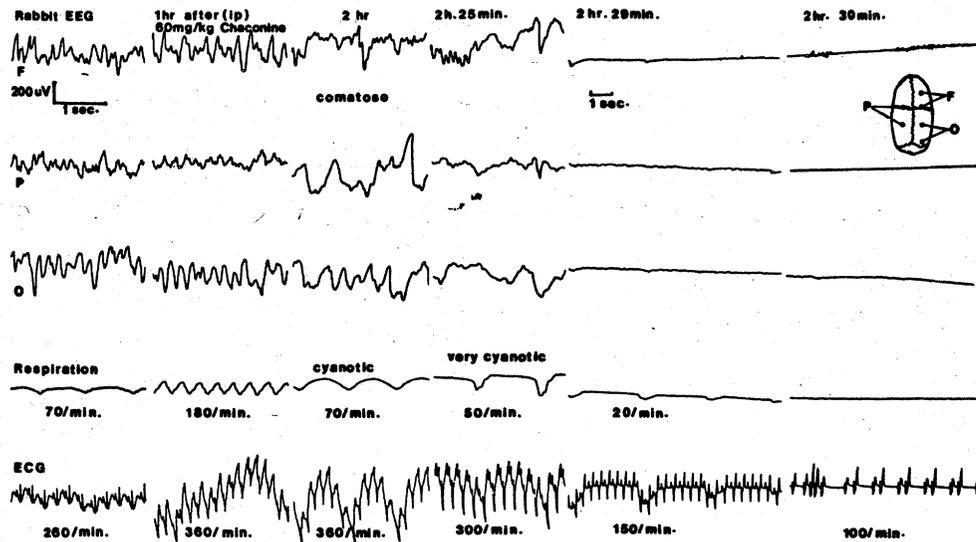
In the rabbit, amounts of test compounds were insufficient for determination of the LD₅₀ values, but we tried to determine the lowest dose of test compound which would kill the rabbit within 8-24 hrs. The lowest lethal doses of α chaconine and α solanine were 50 mg/kg and 40 mg/kg i.p., respectively. Doses of 30 - 100 mg/kg of tomatine were not lethal (Table 2).

Table 2. Lethal Doses in Rabbits(ip)

Glycoalkaloids	mg/kg	death rate
α Chaconine	30,40	0/2
"	50	1/3
"	60	1/1
α Solanine	30	0/1
"	40	1/1
"	50	1/1
Tomatine	30,50,100	0/3

Initially, rabbits receiving lethal doses of α chaconine exhibited no important abnormal EEG patterns(Fig 2), but terminally showed high voltage delta waves which were associated with cyanosis, tachycardia, and coma. The respiratory rate increased from 0 to 60 minutes, and then decreased steadily. Death caused by α chaconine was similar to that caused by α solanine in that the terminal signs began with isoelectric EEG signals followed by respiratory arrest and finally cardiac arrest.

Fig. 2 - Effect of 60 mg/kg ip chaconine on unanesthetized rabbit

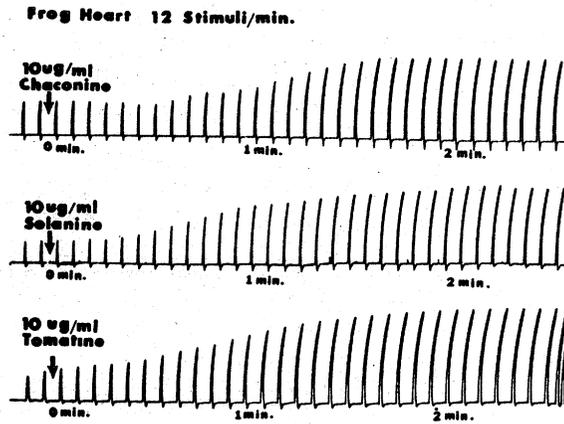


Under our test conditions and over the dose range of 30 to 100 mg/kg (i.p.), tomatine produced neither fatalities in rabbits, nor abnormal EEG signals. With the highest dose (100 mg/kg i.p.), respiratory rate increased over the first hour and then decreased below pre-treatment levels with concomitant increases in heart rate over a 6 hour period.

When administered intravenously (i.v.), at dose levels of 1.0-1.5 mg/kg, α chaconine, α solanine, and tomatine slightly decreased heart rate and blood pressure for a short time. At the level of 2 mg/kg i.v. the glycoalkaloids produced short runs of ventricular extrasystoles and bigeminy within 1-7 minutes after injection.

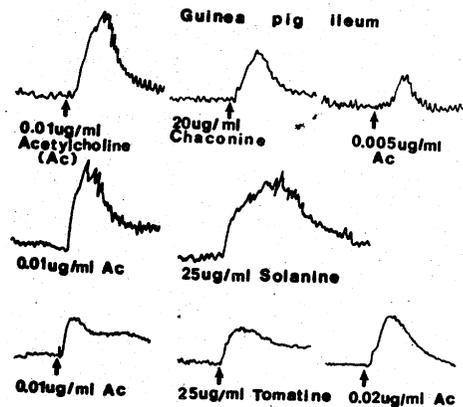
At a dose level of 10 ug/ml, the positive inotropic effects of α chaconine, α solanine, and tomatine on the electrically stimulated isolated frog ventricle (12 stimuli/min) were essentially the same (Fig. 3).

Fig. 3 - Effects of Glycoalkaloids on Isolated Frog Heart



The effects of the 3 glycoalkaloids on guinea pig ileum are shown in Fig. 4. When compared with acetylcholine, α chaconine, α solanine and tomatine were about 570, 520, and 440 times weaker in the ability to produce contractions in 3 different strips of guinea pig ileum.

Fig. 4 - Effects of Glycoalkaloids on Isolated Guinea Pig Ileum



Effects of Glycoalkaloids on the Chick Embryo

The effects of the glycoalkaloids on the hatchability of fertile eggs following yolk sac injection of test compound prior to incubation and at the 96th hour of incubation are shown in Table 3. The embryo toxicity expressed as LD₅₀ was significantly lowered when injections of either test compound or solvent were made prior to incubation. Thus, the yolk sac LD₅₀ for chaconine injected prior to incubation was less than 0.5 mg/kg in comparison with a 96th hour LD₅₀ of 15.5 ± 3.98 mg/kg. The injection of the solvent mixture (propylene glycol + 0.1 N HCL) prior to incubation reduced the hatchability to 1/3 that of the eggs injected at the 96th hour of incubation. Injection of 1.0-1.5 mg/kg of chaconine prior to incubation produced a significant reduction of hatchability when compared with that of solvent control groups. High doses of chaconine injected at the 96th hour of incubation also produced similar reductions in hatchability.

The mean body weights of hatched chicks expressed as a percentage of the corresponding egg weights did not differ significantly in control and treated groups. The decreasing order of hatchability produced by the glycoalkaloids was tomatine, solanine and chaconine.

Although the incidence of congenital malformations produced by the glycoalkaloids was not statistically significant by χ^2 analysis when compared with that of the solvent and absolute control groups, a description of the kinds of abnormalities observed may be in order. In the absolute control group one embryo showed acephaly and the absence of a wing. In the solvent control group one embryo was without upper beak, one had crossed beak and unilateral microphthalmia and one embryo hatched with unilateral exophthalmia. Solanine produced 3 embryos with unfeathered

Table 3. Effects of Glycoalkaloids on Chick Embryo

Glycoalkaloids Dose (mg/kg)	Time of Injection (hour)	No. of Fertile eggs	% Hatched	Body Wt (% egg wt)	Abnormals %
<u>Solanidine</u>					
1.0	0	45	20	70.6	0
<u>Chaconine</u>					
0.5	0	50	28	69.3	0
1.0	0	48	14.5*	68.3	4.1
1.5	0	48	16.7*	69.9	6.25
12.0	96	46	56.6*	69.6	6.5
20.0	96	43	32.5*	66.6	4.6
30.0	96	39	43.5*	68.0	5.1
<u>Solanine</u>					
0.5	0	49	38.7	69.2	0
1.0	0	47	23.4	68.0	8.53
1.5	0	50	28	68.3	2.0
19.0	96.0	45	29.0*	66.6	2.2
<u>Tomatine</u>					
0.5	0	45	31	70.1	0
1.0	0	47	35.5	71.0	0
1.5	0	48	37.5	70.1	0
12.0	96	43	53.4*	68.0	6.9
20.0	96	43	55.8*	69.0	2.3
25.0	96	46	54.3*	68.0	6.5
<u>Solvent Control</u>					
Propylene glycol + 0.1N HCl					
	0	48	33.3	68.8	6.25
	96	43	90.6	69.0	2.3
<u>Absolute Control</u>					
	0	50	88.0	68.8	0
	96	44	86.3	71.0	2.2

*Significantly different from solvent control at the corresponding time of injection ($P < 0.05$).

skin in the retroinguinal area and one with unilateral anophthalmia. Chaconine produced phocomelia in one embryo, a one legged chick, one having only 2 claws on each foot, and one with microcephaly. Tomatine-treated group had one embryo with crossed beak and one with encephalocele. Other malformations seen in control and treated group consisted of inward flexion of toes. Most of the chicks exhibiting malformations died before hatching. Injections of both glycoalkaloids and solvents prior to incubation produced fatalities mostly during the first 3 days and the last 3 days of incubation.

DISCUSSION

Toxicity data from studies of mice, rabbits, and chick embryos demonstrate no essential difference between α solanine and α chaconine. Possibly the β and λ solanines and chaconines might exert a relatively greater toxic effect but these compounds were not available for comparison.

In a similar manner, there were no demonstrable differences between the 2 compounds in the pharmacological responses elicited with respect to positive inotropic effects on the isolated electrically driven frog ventricle contractions of guinea pig ileum and rabbit EEG, ECG, respiration and blood pressure recordings. Tomatine at the highest dose level (100 mg/kg) produced no abnormal EEG recordings in the rabbit, but was similar to α solanine and α chaconine in all other pharmacological effects. From these facts, we presume that other phytoalexins contribute more to the overall toxicity of certain hybrid potatoes than solanine, chaconine and tomatine.

The failure to find significant differences in the incidence of malformations between embryos treated with the three glycoalkaloids and solvent after 0 and 96 hour injection times indicates that these compounds are not teratogenic under the conditions of the experiments. This hypothesis is strengthened by the similar findings in the rat embryo (Ruddick et al., 1974) and the rat, rabbit, and chick embryo (Swinyard and Chaube 1973). These findings do not support the results of (Mun et al., 1975) on the high incidence of malformations in chick embryos injected at 0 and 26 hours of incubation time with observation limited to 72 hours.

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