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Toxicological Effects of α-Solamargine in Experimental Animals

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α-Solamargine isolated from the fresh fruits of *Solanum americanum* Miller was studied for its toxicity. Lethality studies in rats showed a dose-mortality relationship with a LD_{50} of 42 mg/kg body weight intraperitoneally. The chronic and subchronic toxicity investigations indicated that the size of the glycoalkaloid dose was more important than the total glycoalkaloid intake. No appreciable toxic effects were observed at doses below 35 mg/kg body weight as indicated by blood parameters, enzyme levels and histological sections of kidney, liver and cardiac muscle. α-Solamargine did not affect the weight of the testes and epididymis or the number of spermatozoa but produced a slight irritation and congestion in the epididymis and testis at doses up to 50 mg/kg body weight. Copyright © 2003 John Wiley & Sons, Ltd.

Keywords: *Solamargine*; *Solanaceae*; glycoalkaloids; toxicology; pharmacology.

INTRODUCTION

The *Solanaceae* family include many species of plants important to man as diverse agricultural crops and medicinal treatments (Fewell *et al*., 1994; Cham and Meares, 1987; Cham *et al*., 1987; Chataing *et al*., 1996). The primary components are steroidal alkaloids of the spirosolane or solanidane types, which generally occur as glycosides (Fig. 1). Toxicological studies indicate that the solanidanes seem to be more toxic than their corresponding spirosolanes – α-solamargine, solasonine and solasodine (Friedman and McDonald, 1997). However, the toxicological profile has been centred upon solanine, α-chaconine and other potato components. Also, most of the alkaloids from the Solanaceae inhibit acetylcholinesterase activity (Roddick, 1989). The teratogenic effects of these alkaloids are inconclusive (Friedman and McDonald, 1997). In animals, adverse physiological effects of steroidal glycoalkaloids are manifested in a number of ways, such as reduced respiratory activity or blood pressure, bradycardia and haemolysis which are thought to stem mainly from membrane disruption, inhibition of acetylcholinesterase activity or interference with sterol/steroid metabolism or a combination (Roddick, 1989; Kusano *et al*., 1987).

In this work, experiments are reported that test some toxicological properties of solamargine and compare them with data reported for other solanum alkaloids such as solanine and glycoalkaloids present in food consumed by humans.

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MATERIALS AND METHODS

Animals. The recipients, 6 to 8 week-old male Wistar rats with a body weight of approximately 150–200 g, were purchased from the Animal House Center of La Universidad de Los Andes.

Solamargine isolation. Fresh fruits (770 g) 1–2 weeks old of *Solanum americanum* Miller were harvested from the Botanical Garden at the Pharmacy Faculty, The University de los Andes. They were washed with 250 mL of distilled water, minced finely and blended. After boiling the mixture for 5 min, 500 mL glacial acetic acid (5% total volume) and 500 mL of methanol were added and left at room temperature for 3 days. The mixture was filtered, the filtrate was heated at 70°C, and the alkaloids precipitated with NH_4OH at pH 12. The solids were resuspended in 5% acetic acid and reprecipitated with NH_4OH at pH 12. The crude alkaloids (1.15 g) were washed with cold water, dried at 45°C, and dissolved in MeOH. The glycoalkaloids were chromatographed on an open silica gel column which was eluted with $CHCl_3$/MeOH/H_2O mixtures starting with 140:10:1, and 100 mL fractions were collected. Fractions 37–46 eluted with $CHCl_3$/MeOH/H_2O 90:10:1 afforded 128 mg of solasonine and fractions 69–100 eluted with the same solvent mixture afforded 152 mg of solamargine. The fractions were inspected by TLC on silica gel plates using $CHCl_3$MeOH/1% NH_4OH (2:2:1, lower phase) as solvent. Solasonine and solamargine showed R_f 0.32 and 0.22, respectively. Analysis of the samples by HPLC, using a μ-Bondapak CN column revealed the existence of two main peaks which correspond to solamargine and solasonine. The smaller peaks correspond to mono- and di-glycosides of solasonine.

The identity of the glycoalkaloids was confirmed by ^{13}C-NMR spectra by comparison with values reported

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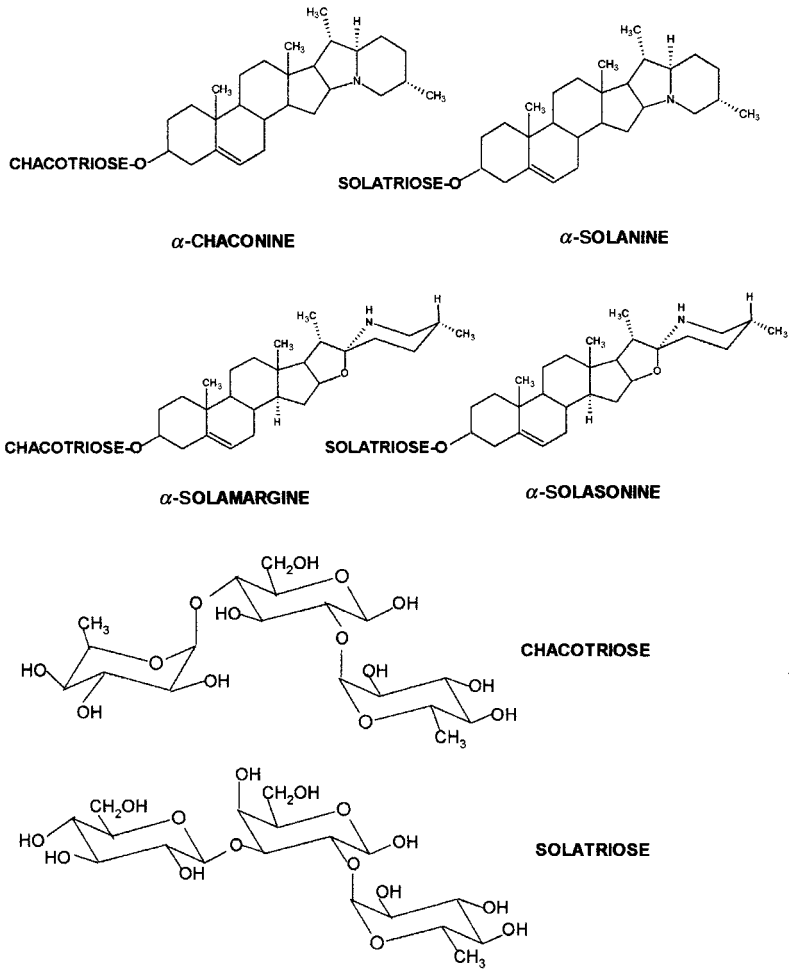

Figure 1. Structure of *Solanaceae* glycoalkaloids.

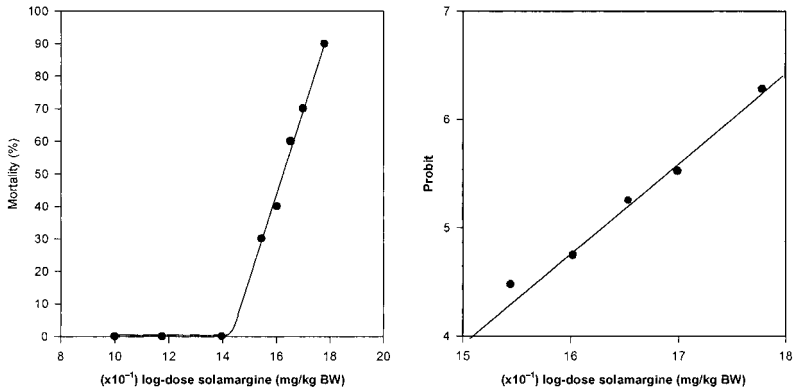

Figure 2. Determination of lethal dose 50 (LD_{50}).

by Mahato *et al.* (1980). The glycoalkaloid was dissolved in 0.1% acetic acid and 0.9% NaCl, filtered, and sterilized until its inoculation to the recipient animals.

Lethal dose 50 (LD_{50}). Ninety Wistar rats, differing less than 25 g in weight, were randomly divided into nine groups of ten individuals each. Single doses of solamargine, ranging from 10 to 60 mg/kg body weight (BW), were administered intraperitoneally (i.p.) to the rats. As a control group 10 Wistar rats were inoculated with a single i.p. dose of solvent. All groups were watched each 3 h for 24 h, and the number and the percentage of deaths in each group were calculated. The percentages were converted to probit and the LD_{50} was calculated from a statistical linear regression analysis which include the standard deviation (S.D.).

Acute toxicity. A total of 20 Wistar rats were injected i.p. with doses of 10 mg/kg BW α-solamargine over 5 continuous days. A group of 10 animals served as a control.

Subchronic toxicity. A total of 50 Wistar rats were separated into groups of 10 rats each. Two groups served as the control by inoculation of anaesthesia and

Table 1. Effect of α-solamargine on blood cells constituents, creatinine and enzyme levels

Treatment	RBC (x10 cell/mm^3)	PCV (%)	Hb (g/dL)	MCV (fL)	MCH (Pg)	GOT (U/mL)	GTP (U/mL)	Creatinine (mg/dL)	AP (U/mL)
Control (anaesthesized only)	57 ± 4	41 ± 3	13.5 ± 0.8	73 ± 4	24 ± 1	177 ± 4	46 ± 1	0.74 ± 0.03	33 ± 7
Control (solvent)	49 ± 4	35 ± 3	11.5 ± 1.0	71 ± 2	23 ± 1	165 ± 6	52 ± 3	0.47 ± 0.01	35 ± 4
Solamargine 15 mg/kg BW	57 ± 3	41 ± 2	14.4 ± 0.3	74 ± 2	26 ± 1	173 ± 3	41 ± 2	0.56 ± 0.09	40.5 ± 0.5
Solamargine 25 mg/kg BW	54 ± 2	3(S)5 ± 1	11.9 ± 0.6	65 ± 3	22 ± 1	178 ± 4	47 ± 7	0.48 ± 0.05	44 ± 2
Solamargine 35 mg/kg BW	60 ± 4	42.3 ± 0.3	14.6 ± 0.7	72 ± 5	25 ± 2	215 ± 1	138 ± 4	0.53 ± 0.07	63 ± 4

Data shown are mean ± SD, $n = 10$. Significant differences were determined with respect to the control (anaesthesized only). $p < 0.01$ was considered statistically significant. Data shown indicated non statistical significance, except for GTP and AP.
RBC, red blood cell count; PCV, packed cell volume; Hb, haemoglobin estimation; MCV, mean cell volume; MCH, mean corpuscular haemoglobin; GOT, glutamate-oxaloacetate transaminase; GTP, glutamate-pyruvate transaminase; AP, alkaline phosphatase.

solvent, respectively, and the other three groups were injected i.p. with either 15, 25 or 35 mg/kg BW of α-solamargine. The animals were observed for general symptoms of toxicity, external symptoms and mortality. All animals were killed 24 h after the last exposure. Vital organs (liver, kidney, spleen, cardiac and skeletal muscle, testes) were extracted and sampled for histological analysis. Tissues were fixed in Bouin's solution, dehydrated in alcohol and embedded in paraffin. Microscopic sections were stained with haematoxylin and eosin. Blood was isolated from the rats and analysed for haemoglobin estimation (Hb), packed cell volume (PCV), mean corpuscular haemoglobin (MCH), mean cell volume (MVC) and red blood cell count (RBC). Levels of creatinine (CREA), glutamate-oxaloacetate transaminase (GOT), glutamate-pyruvate transaminase (GTP) and alkaline phosphatase (AP) were also determined.

Spermatogenesis. Male Wistar rats were inoculated i.p. with doses of α-solamargine of 15, 25 and 35 mg/kg BW. The rats were killed 24 h after the last exposure and the epididymes of rats were isolated to determine the number of spermatozoa. Also, sections of the right and left testis were isolated to determine their weight and histology.

Statistical treatment of data. Data were analysed by a statistical non-parametric Kruskal–Allis ANOVA test with the Statistica 6.0 program. Application of the test was used to determine the statistical significance of data. $p < 0.01$ was considered statistically significant.

RESULTS

Toxicity test

Lethality studies showed a dose-mortality relationship which was apparently sigmoidal. A plot of probit values (% mortality) versus log-dose of α-solamargine gave a straight line (Fig. 2). From the straight line graph, the LD_{50} value of solamargine was extrapolated. This value was about 42 ± 2 mg/kg BW i.p.

No animal died earlier than 6 h. However, the high dose rats (over 60 mg/kg BW) died within 3 h after a single i.p. injection. Otherwise, multiple i.p. doses of 10 mg/kg animal weight of α-solamargine administered over 5 consecutive days did not cause any mortality or difference in the body weight of the animals in that period. This observation confirmed the results of Chaube and Swinyard (1976) that the dose size seems to be more important than the total glycoalkaloid intake. Also observed were muscular contraction and dizziness at the initial 2 h after α-solamargine inoculation, an effect also observed by Aldous *et al.* (1980) in rats injected i.p. with α-chaconine. No bleeding from the eyes or nose was observed.

Subchronic toxicity

The blood of Wistar rats inoculated with α-solamargine was analysed by blood components. Chemical determination of GOT, GTP, creatinine and AP indicated no

Table 2. Testes, epydidimis and spermatozoa analysis of Wistar rats treated with different α-solamargine doses

Treatment	Right testicle weight (g)	Left testicle weight (g)	Right epidydimis (g)	Left epidydimis (g)	Number of spermatozoa (x10^8 spermatozoa/tissue weight)
Control (anaesthesized only)	0.76 ± 0.04	0.76 ± 0.05	0.05 ± 0.00	0.05 ± 0.00	0.90 ± 0.10
Control (solvent)	0.75 ± 0.11	0.77 ± 0.14	0.05 ± 0.01	0.05 ± 0.01	0.90 ± 0.10
Solamargine 15 mg/kg BW	0.82 ± 0.08	0.83 ± 0.09	0.05 ± 0.00	0.05 ± 0.00	0.90 ± 0.10
Solamargine 25 mg/kg BW	0.90 ± 0.08	0.90 ± 0.04	0.07 ± 0.02	0.07 ± 0.01	0.93 ± 0.15
Solamargine 35 mg/kg BW	0.85 ± 0.25	0.91 ± 0.29	0.06 ± 0.02	0.06 ± 0.02	0.90 ± 0.20
Solamargine 45 mg/kg BW	0.98 ± 0.12	0.76 ± 0.39	0.06 ± 0.01	0.07 ± 0.01	0.94 ± 0.10
Solamargine 50 mg/kg BW	0.97 ± 0.09	0.98 ± 0.07	0.06 ± 0.00	0.06 ± 0.00	0.86 ± 0.20

Data shown are mean ± SD, $n = 10$. Significant differences were determined with respect to the control (anaesthesized only). $p < 0.01$ was considered statistically significant. Data shown indicated non statistical significance.

appreciable differences in values, except for a slight, but not significant, increase in these variables measured at doses of 35 mg/kg BW (Table 1). Examination of the vital organs tissues did not depict any toxic effect (data not shown).

Spermatogenesis analysis

α-solamargine at doses of 15, 25 and 35 mg/kg BW did not affect the number of spermatozoa or the weight of the testicle and epididymis (Table 2). Examination of the testes at doses 45 and 50 mg/kg BW, respectively, indicated subcapsular congestion possibly produced by hyperaemia. Similarly, epididymis at the same doses showed a difuse congestion.

DISCUSSION

Most studies of glycoalkaloids of *Solanaceae* have been centred on rats, mice, hamster and rabbits (Friedman and McDonald, 1997). The toxicological properties of α-solamargine, a glycoalkaloid with biological activity against *Herpes simplex* I, *Herpes zoster* and *genital Herpes* (Chataing *et al*., 1996) and *Trypanosoma cruzi* (Chataing *et al*., 1998) has been tested. This glycoside has a toxicity range in the order of other *Solanaceae* glycoalkaloids considering its LD_{50} value which is similar to those obtained for solanine (Nishie *et al*., 1975) and BEC mixtures in rats (Cham *et al*., 1991). A similarity of LD_{50} values on rats was observed in comparison with values determined in mice. However, the studies performed with solanaceae alkaloids have revealed differences in LD_{50} values depending on the experimental animal used, the conditions of the assays and the purity of the alkaloids. The LD_{50} i.p. for α-solanine, α-chaconine and tomatine in mice were 27, 30 and 34 mg/kg BW, respectively and for most animals, the i.p. LD_{50} of the various glycoalkaloids were around 30 to 60 mg/kg BW (Friedman and McDonald, 1997).

Although the cause of death in the high dose recipient animals could be attributed mainly to acetylcholinesterase (AchE) inhibition and cell membrane disruption, these effects do not appear to be pronounced in the case of α-solamargine. In effect, Roddick (1989) reported slight AchE inhibition by this glycoalkaloid, which confirms the importance of the aglycone moiety in the AchE activity. The absence of bleeding in the eyes and nose, together with the histology which did not show renal and hepatic congestion, indicated a low effect on cell membrane disruption of α-solamargine in doses below the LD_{50} value. In contrast, even though the number of spermatozoa were not affected at the doses administered congestion of the epididymis and testicle tissues was observed at doses of 45 and 50 mg/kg BW.

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