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Research Paper

Effect of *Solanum paniculatum* leaf extract on food consumption, fertility and carbohydrate metabolism of *Bradybaena similaris* snail.

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ABSTRACT

The objective of this study was to investigate the effects of the LC_{50} of an aqueous leaf extract of *S. paniculatum* on the food consumption, fecundity and carbohydrate metabolism of *B. similaris.* For this purpose, adult snails were exposed to the LC_{50} of the aqueous extract for 24, 48 and 72 hours. The food consumption only declined in the last weeks of observation, while there was a significant reduction in fecundity. Also, the exposed snails had an increase in free glucose in the hemolymph and of glycogen in the cephalopedal mass, with concomitant reduction of glucose in the digestive gland and also of galactogen in the albumen gland. Finally, there was a significant increase in the lactate dehydrogenase activity in the snails exposed to the LC_{50} of *S. paniculatum* in the first 24h after exposure, showing acceleration of the anaerobic metabolism in this period, possibly due to the effects of intoxication. The reduction fecundity of exposed snails can be related to their redirection of reserves for detoxification and survival. The maintenance of normal food intake appears not to have guaranteed normal metabolic functions, since it did not prevent the decline in fecundity. The results therefore show that the extract of this plant causes physiological changes in the snails, with a negative effect on fecundity, indicating partial castration by redirection of nutrients that otherwise would be used for reproduction.

Keywords: Carbohydrates; Fecundity; Plant molluscicides.

INTRODUCTION

The Asian snail, *Bradybaena similaris*, is host to helminths and an important pest of various crops (Araújo, 1989; Araújo and Bessa, 1995; Pinheiro and Amato, 1994). Therefore, control of populations of this species is necessary. The molluscicides currently used for this purpose have limitations due to their low specificity and high cost and toxicity (Jurberg, et al., 1989). Because of these drawbacks of chemical

substances, the World Health Organization (WHO, 1965; 1983) encourages research to find substances of plant origin for control of these pests. In Brazil, studies of plant molluscicides have mainly concentrated on control of freshwater snail species hosts of *Schistosoma mansoni* (Sambom, 1907) and *Fasciola hepatica* (Jurberg et al., 1989; Mendes, et al., 1997; Vasconcelos and Amorim, 2003a; 2003b). Very little research has been conducted on the control of land snails.

Brazil has a rich flora of species with potential pharmacological activity. Many plants used in popular medicine against infections and parasitoses have been shown to have biocidal properties without posing harmful side effects to human health (Brito and Brito, 1993). The Solanaceae family contains many species with varied toxicity levels, of which *Solanum paniculatum* stands out for its application in phytotherapy due to its proven bactericidal, fungicidal and molluscicidal effects. Its biocidal effects are related to chemical composition of the plant, among wich the presence of tannins, flavonoids and saponins found in all parts of the plant (Xavier, et al., 2010; Chinedu, et al., 2011; Chinthana and Ananthi, 2012; Silva, et al. 2012).

Besides immediate lethality to snails, intoxication by plant extracts can directly interfere in the animals' metabolism, causing a reduction in their fecundity (Silva, et al., 2012). Studies have desmonstrated tha plant extracts can cause alteration in glycemic levels, reducing energy reserves designed to reproduction (Mello-Silva, et al., 2007; 2010). This fact can make these extracts efficient for use in snail control programs by reducing the future population density (Silva, et al., 2012).

Our aim was to investigate the reproductive alterations caused by exposure to the LC_{50} of an aqueous extract of *S. paniculatum* and *S. lycocarpum* on adult *B. similaris* specimens. Also, the changes in food consumption and in the levels of glucose in the hemolymph, glycogen in the digestive gland and cephalopedal mass and galactogen in the albumen gland as well as of the lactate dehydrogenase activity in the exposed snails were analyzed.

MATERIALS AND METHODS

Snails: The animals used in this study were bred at the Laboratório de Biologia de Moluscos of Museu de Malacologia Prof. Maury Pinto de Oliveira, Universidade Federal de Juiz de Fora (UFJF) (Juiz de Fora, Minas Gerais, Brazil), where the reproductive biology experiments were also conducted.

Collection and preparation of the plant material to make the extracts: New *S. paniculatum* leaves measuring approximately 18cm² lengths, without injuries caused by animals, were collected in São Pedro district of the Juiz de Fora City in May 2011. A dried specimem was prepared and identified in Leopoldo Krieger Herbarium, UFJF. The leaves were washed and dried under natural temperature conditions (24±4°C) for two weeks and then ground into powder, after which the material was kept in vials under refrigeration. The sublethal concentration (LC_{50}) of the aqueous extract was prepared according to Silva, et al. (2012).

Changes in the food consumption of adult Bradybaena similaris specimens exposed to the LC_{50} of S. paniculatum: To evaluate the effect of exposure to the LC_{50} on the food consumption of *B. similaris*, 30snails (10animals/group) were exposed to 15ml of the extract, which was sprayed directly on them in polyethylene terrariums (250ml capacity) containing 10g of sterilized earth. The snails remained in direct contact with the extract for 24, 48 and 72 hours. After exposure, the animals were transferred to other terrariums containing the same kind of earth moistened with tap water (Silva, et al., 2012). Each group was offered 2g of a mixture of commercial poultry ratio and

calcium carbonate (Araújo and Bessa, 1995) in plastic flasks. Every two days for four weeks the remaining food was weighed to calculate the consumption. The control groups were submitted to the same procedures except they were sprayed with distilled water instead of the extract. Another control was used without snails to evaluate the moisture retention of the feed so as not to overestimate the consumption (Meireles, 2009).

Analysis of changes in the fecundity of Bradybaena similaris adults exposed to the LC_{50} of S. paniculatum: To assess the fecundity changes, 90 snails (10snails/group) were exposed to 15ml of the extract for 24, 48 and 72h, with three repetitions for each period (Silva, et al., 2012). The control groups were placed in similar terrariums and received the same quantity of distilled water. After each exposure period, the snails were transferred to other terrariums containing sterilized earth moistened with tap water as a substrate (Silva, et al., 2008) and were fed according to Bessa and Araújo (1995) up to four weeks.

During this period, the number of postures and eggs produced by the exposed and unexposed snails was counted. The fecundity was calculated as the average number of eggs/live snail for each exposure period.

***Changes in the carbohydrate metabolism of Bradybaena similaris exposed to the LC_{50} of Solanum paniculatum*:** The biochemical analyses were performed at the Laboratório de Biofísica, Departmento de Ciências Fisiológicas, Instituto de Biologia, Universidade Federal Rural do Rio de Janeiro, Seropédica, Rio de Janeiro State.

To analyze the changes in the glucose concentration, 120 adult snails were exposed to the extract according to the method described previously during 24, 48 and 72 hours. The control group of 120 snails was exposed to distilled water during the same periods.

After each exposure interval the hemolymph was collected by cardiac puncture. The glucose concentration was determined by adding 10ml of serum to a medium containing 0.05M of sodium phosphate buffer solution (pH 7.45), 0.03mM of aminoantipyrine, 15mM of p-sodium hydroxybenzoate and at least 12kU of glucose oxidase and 0.8KU of peroxidase per liter. The absorbance was read in a spectrophotometer at a wavelength of 510nm against a reaction blank using 100 mg/dL of D-glucose as a standard (Doles Reagentes®).

The hemolymph collected was also used to determine the lactate dehydrogenase (LDH) activity (E.C. 1.1.1.27/E.C. 1.1.1.28) by the addition of 25µL of hemolymph to 1mL of a substrate containing a solution of 0.1M of lactate, 0.005M of o-phenanthroline in 0.2M of Tris (pH 8.8) and a drop of a solution of 0.012M of ammonium ferric sulfate, after which the mixture was incubated at 37ºC for 2 minutes. After this time, a drop of a solution was added containing 130mg of NAD and 4mg of phenazine methosulfate, after which the mixture was again incubated at 37ºC for 5 minutes. After this period, 1mL of a stabilizing solution of 0.5M of hydrochloric acid was added. The absorbance was read in a spectrophotometer at a wavelength of 510nm against a reaction blank and standard solution of LDH containing 350U.I/L of LDH. The results were expressed as µmol of NADH/L of hemolymph/minute.

For determinations glycogen and galactogen contents, 90 snails (30 snails after each time interval) were removed from their shells and dissected in physiological solution under a stereoscopic microscope to obtain the albumen gland, digestive gland and cephalopedal mass. A pool of tissues was obtained weighing 1g by fresh weight, which was processed for quantification of the polysaccharides.

To obtain the precipitates of glycogen and galactogen, the procedures were followed as proposed by Pinheiro and Gomes (1994) and the quantification was performed in a spectrophotometer with 3,5-dinitro-salicylate (3,5 DNS) (Sumner, 1925), with reading of the absorbance at a wavelength of 535 nm. The results were calculated by the Lambert-Beer Law, based on at least three coherent readings, and expressed as grams of glucose or galactose per gram of tissue (fresh weight).

Data analysis: For comparison between exposed/unexposed snails regarding food consumption, fecundity, concentration of carbohydrates (glucose, glycogen and galactogen) and LDH activity, ANOVA (F) was used, followed by the Tukey test (Q), using the BioEstat version 5.0 programs. Simple linear regression was used to assess the relationship of the results found and the time of exposure to the extracts.

RESULTS

Changes in the food consumption: Significant reduction in food intake was only observed for the snails exposed for 24 hours (here Tukey test was used. Q=10.2; *P*=0.03) and 48 hours (Q=12.1; *P*=0.03) in the third week after exposure and for those exposed for 72 hours (Q=10.9; *P*=0.03) in the fourth week after exposure (Table I). Também não houve variação do consume qundo comparamos os períodos de exposição (F=1.0; *P* =0.45) (Table 1).

Changes in the fecundity: The fecundity of *B. similaris* exposed to the LC_{50} of the *S. paniculatum* extract was significantly lower than that of the unexposed snails (*P*<0.01). The reduction percentages at the end of the observation period were 75%, 93% and 79% for the groups exposed for 24, 48 and 72 hours, respectively (Table 2). There was no statistical difference between the fecundity of unexposed snails and snails exposed to the extract during the observation time (Table 2).

Changes in the carbohydrate metabolism: The concentration of glucose in the hemolymph of the snails exposed to the LC_{50} of *S. paniculatum* after 24 hours of exposure increased by 35% (Q= 3.76; *P*<0.01) and by 23% after 48 hours of exposure (Q= 2.7; *P*<0.05), while after 72 hours of exposure the increase was only 10% (Q= 1.0; *P*=1.9) (Table 3). There was no significant correlation between the variation in the glucose concentration and the exposure time (F=0.11; *P*=0.79; R^2=0.10).

The exposure to the *S. paniculatum* extract caused a reduction of 78.70% in the glycogen concentration in the digestive gland after 48 hours (Q=66.0; *P*=0.002) and of 96.60% after 72 hours (Q=37.8; *P*=0.004), while after 24 hours there was an increase of 7%, although this increase was not statistically significant (F=0.89; *P*=0.59) (Table 3). There was a negative correlation between the glycogen concentration in the digestive gland and time of exposure (F=540.0; *P*=0.02; R^2=0.99) (Table 3).

The glycogen concentration in the cephalopedal mass declined by 194% (Q=10.1; *P*<0.01) after 24 hours of exposure, by 1245% (Q=16.4; *P*<0.01) after 48 hours and by 83% (Q=16.4; *P*<0.01) after 72 hours (Table 3). However, in all cases there was no significant correlation between the glycogen concentration in the cephalopedal mass and exposure time to the LC_{50} of *S. paniculatum* (F=0.62; *P*>0.05; R^2=0.38).

Exposure to the LC_{50} of *S. paniculatum* caused a reduction in the galactogen concentration in the albumen gland of 19% after 24 hours, but this reduction was not significant (*P*>0.05). The declines were 175% (Q=5.7; *P*<0.05) after 48 hours and 89% (Q=7.14; *P*<0.01) after 72 hours (Table 3). There was no significant correlation

between the reduction of the galactogen concentration and time of exposure to the LC_{50} of *S. paniculatum* (F=0.48; *P*=0.62; R^2=0.32).

The mean LDH activities were 5.91±1.08, 1.09±0.26 and 2.72±0.20 (1mmol of NADH/L of hemolymph/minute) exposed to the extract for 24, 48 and 72 hours, respectively. For unexposed snails the mean enzymatic activity was 0.74±0.02, 1.30±0.21 and 1.86±0.24 (1mmol of NADH/L of hemolymph/minute) the same intervals of exposure. Therefore observed a significant increase (698%) in the LDH activity after exposure for 24 hours to the extract (Q=5.74; *P*<0.05) while after 48 hours there was a reduction (17%), which was not statistically significant (*P*>0.05) in relation to the control group. After 72 hours there was another increase in activity, of 46% (Q=5.60; *P*<0.05) (Figure 1). There was no relation between the variation of the LDH activity and the exposure time (F=0.47; *P*=0.62; R^2=0.32).

DISCUSSION

The results above indicate that the changes in the reproductive biology of the exposed snails are possibly related to alterations in the carbohydrate metabolism. Similar to findings of this study, Mello-Silva et al., (2007) observed a reduction of fecundity of *Biomphalaria glabrata* (Say, 1818) infected with *S. mansoni* when exposed to *Euphorbia splendens var. hislopii*. The authors obtained similar results when compared to those observed in the present study, i.e., an increase in the levels of glucose in the hemolymph and cephalopedal mass and a reduction in the glycogen reserves in the digestive gland, indicating increased energy consumption and acceleration of glycolysis (Mello-Silva, et al., 2010).

A reduction of fecundity of *B. similaris* due to exposure of *Subulina octona* (Bruguiére, 1789) to the LC_{50} of *S. paniculatum* was reported by Silva, et al. (2012). The authors observed that besides reduced fecundity, the exposure to successive applications of this extract caused alterations in the offspring produced.

Similar to the present findings, Lustrino, et al. (2009) observed an increase in the glycogen concentration in the cephalopedal mass of *B. similaris* exposed to a 5% concentration of an extract prepared from *Alamanda catharthica* (Apocynaceae) seeds. However, those authors found an increase in the concentration of this polysaccharide in the tissues of the digestive gland. This suggests different mechanisms of action of these two plants.

Reductions in the carbohydrate reserves in the digestive gland due to exposure to plant molluscicides have been reported for *Achatina fulica* Bowdich, 1822 (Oliveira, 2007) and *B. glabrata* (Mello-Silva, et al., 2010) (both exposed to the latex of *Euphorbia splendens var. hislopii*). The exposure of *glabrata* to a crude extract of *Solanum malacoxylon* (Sendter) also caused a reduction in the glycogen content of the digestive gland and an increase in the free glucose concentration in the hemolymph, as found in the present study (Mello-Silva, et al., 2006).

The reduction of the galactogen content can be directly related to the reduced fecundity. Galactogen is a reserve used as an energy source for formation of the perivitelline fluid which provides nutrition to the embryo during development (Tompa, 1984). However, that reserve can be reduced by reasons other than egg production. Under severe metabolic stress conditions, such as long periods of estivation or parasitism (Pinheiro and Amato, 1994; Pinheiro, 1996), the snail stops synthesizing galactogen from glucose, since the available carbohydrates are directed to supply the increased energy demand in response to the stress. In this case, the reduction in the galactogen concentration occurs not because of its increased

consumption, but rather due to a reduction of synthesis. As observed in this study, the exposure to the extract caused a metabolically unfavorable situation, inducing reduction of this reserve.

Unlike observed in this study, Lustrino, et al. (2009) observed that exposure of *B. similaris* to an extract of *A. cathartica* caused an increase in the galactogen concentration. The authors suggested that this increase could be related to the acceleration of the synthesis of this polysaccharide or interruption/reduction of the reproductive process as a way to assure a compensatory mechanism in the future. However, the authors did not evaluate the effects on the snails' fecundity.

Glycemia in snails is precisely regulated (Thompson and Lee, 1986). The upsetting of the glucose homeostasis is an indication of intoxication of the animal. The intoxication of the snails by exposure to the extract might have caused a change in the neuroendocrine system, responsible for regulating glycemia (light green cells [LG]). The detoxification of the organism raises the energy consumption, resulting in depletion of these deposits.

The clear transfer of energy from the digestive gland to the muscles and also the release of free glucose in the hemolymph observed in the groups exposed to the LC_{50} of *S. paniculatum* demonstrate the metabolic compensation effect for regulation of homeostasis.

The exposure to the LC_{50} of *S. paniculatum* did not interfere in the food consumption with the significant reduction only occurring in the final period of observation. However, maintenance of normal food consumption possibly did not assure maintenance of carbohydrate reserves in the organism, because it did not prevent a sharp reduction in fecundity during the same period.

There was only a significant increase in the LDH activity of *B. similaris* after exposure to the LC_{50} of *S. paniculatum* for 24 hours. Likewise, Tripathi and Singh (2004) found an increase in this enzyme activity in *Lymnaea acuminata* exposed to a sublethal concentration of the pesticide carbaryl. This enzyme is related to the balance between anabolism and catabolism of carbohydrates and the conversion between pyruvic and lactic acids (Abston and Yarbrough, 1976). The results obtained demonstrate that in the 24 hours after exposure there was acceleration of anaerobic metabolism, possibly due to the effects of the intoxication, as found by Rao, et al. (2003).

The fecundity reduction of snails exposed to plant molluscicides can be related to other metabolic pathways. Rao and Singh (2000) noted a reduction in fecundity induced by exposure of the species *A. fulica* to different combinations of plant derivatives (*Azadirachta indica*, *Cedrus deodara*, *Allium sativum* and *Nerium indicum* in different proportions). They also observed reductions in the content of DNA, RNA and phospholipids and a concurrent increase in the activity of lipid peroxidase in the ovotestis of the snails, indicating impaired production of gametes.

CONCLUSION

It can be conclude that *S. paniculatum* extract caused negative effects in glycemic metabolism of *B. similaris* leading to an acceleration of anaerobic metabolism. It is possible that the damage in glycemic metabolism contributed to snail's castration observed after the exposure to the plant extract. The results therefore demonstrate that the extract of *S. paniculatum* caused physiological changes in the snails, negatively affecting their fecundity through a process of partial castration due to the diversion of nutrients that otherwise would have been used for reproductive activity.

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Table-1: Mean food consumption (g) by *B. similaris* in response to exposure to aqueous extract of *Solanum paniculatum* leaves for 24, 48 and 72 hours, observed during four weeks.

Weeks	Exposure Time (hours)					
	24		48		72	
	Control	Exposed	Control	Exposed	Control	Exposed
1	$0.20\pm0.03^{a,A}$	$0.15\pm0.12^{a,A}$	$0.17\pm0.06^{a,A}$	$0.15\pm0.10^{a,A}$	$0.45\pm0.2^{a,A}$	$0.09\pm0.08^{a,A}$
2	$0.36\pm0.1^{a,A}$	$0.14\pm0.15^{a,A}$	$0.7\pm0.5^{a,A}$	$0.2\pm0.1^{a,A}$	$0.6\pm0.3^{a,A}$	$0.3\pm0.2^{a,A}$
3	$0.60\pm0.2^{a,A}$	$0.2\pm0.2^{b,A}$	$0.86\pm0.2^{a,A}$	$0.22\pm0.2^{b,A}$	$0.7\pm0.3^{a,A}$	$0.46\pm0.2^{a,A}$
4	$0.50\pm0.25^{a,A}$	$0.21\pm0.1^{a,A}$	$0.6\pm0.18^{a,A}$	$0.25\pm0.13^{a,A}$	$0.63\pm0.19^{a,A}$	$0.1\pm0.09^{b,A}$
MM±SD	0.41±0.21	0.18±0.14	0.56±0.24	0.21±0.11	0.56±0.25	0.24±0.14

- a = indicate differences between the control and treated groups in each exposure interval; A = indicate differences between the control and treated groups over time (ANOVA; $P<0.05$). (MM±SD=Monthly Mean± standard deviation).

Table-2: Mean fecundity (number of eggs/live snail) of *Bradybaena similaris* in response to exposure to aqueous extract of *Solanum paniculatum* leaves for 24, 48 and 72 hours.

Weeks	Exposure Time (hours)					
	24		48		72	
	Control	Exposed	Control	Exposed	Control	Exposed
1	$8.3 \pm 5.4^{a,A}$	$4.7 \pm 3.3^{b,A}$	$7.0 \pm 3.9^{a,A}$	-	$11.1\pm4.3^{a,A}$	$0.9 \pm 1.2^{b,A}$
2	6.6 ± 2.4	-	$8.7 \pm 2.5^{a,A}$	$2.5 \pm 3.5^{b,A}$	$6.3 \pm 2.6^{a,A}$	$3.5 \pm 2.6^{b,A}$
3	$6.3 \pm 3.5^{a,A}$	$0.9 \pm 1.2^{b,A}$	$6.8 \pm 1.8^{a,A}$	$0.5 \pm 0.7^{b,A}$	$6.9 \pm 2.6^{a,A}$	$2.6 \pm 2.0^{b,A}$
4	$6.4 \pm 2.5^{a,A}$	$2.5 \pm 1.5^{b,A}$	$5.5 \pm 3.3^{a,A}$	-	$8.1 \pm 4.0^{a,A}$	-
MM±SD	6.9 ± 3.40	2.0 ± 1.50	7.0 ± 2.87	0.75± 1.05	8.1 ±3.40	1.8 ± 1.45.

- Footnotes are same as shown in table-1.

Table-3: Mean of glucose (mg/dL), glycogen in digestive gland, glycogen in cephalopedal mass and galactogen (g of glucose/g tissue, of *Bradybaena similaris* in response to exposure to aqueous extract of *Solanum paniculatum* leaves for 24, 48 and 72 hours.

Weeks	Exposure Time (hours)					
	24		48		72	
	Control	Exposed	Control	Exposed	Control	Exposed
Glucose	6.79 ±0.5	10.6±0.10	8.2±1.20	10.6±0.81	9.0±1.8	10.0±0.3
Glycogen DG	$2.70\pm0.02^{a,A}$	$2.90\pm0.30^{b,A}$	$2.80\pm0.40^{a,A}$	$1.60\pm1.0^{b,B}$	$2.90\pm0.18^{a,A}$	$1.50\pm0.3^{b,C}$
Glycogen CM	$0.6 \pm0.07^{a,A}$	$0.20\pm0.01^{b,A}$	$0.11\pm0.01^{a,B}$	$1.37\pm0.15^{b,B}$	$0.16\pm0.01^{a,A}$	$0.93^{a,A}\pm0.08^{b,C}$
Galactogen	$0.6.3\pm0.0^{a,A}$	$0.50\pm0.08^{a,A}$	$0.44\pm0.04^{a,A}$	$0.16\pm0.09^{b,A}$	$1.7\pm 0.2^{a,B}$	$0.90\pm0.2^{b,B}$

- *Small letters indicate differences between the control and treated groups in each exposure interval; capital letters indicate differences between the control and treated groups over time (ANOVA; $P<0.05$).

