Anthelmintic study of *Solanum lycocarpum* St. Hill in mice naturally infected with *Aspiculuris tetraptera*

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Abstract: This study intends to add new data on the helminthes parasites of laboratory mice. It has been investigated the anthelmintic activity of *Solanum lycocarpum* (*Solanaceae*) extracts against *Aspiculuris tetraptera* in mice naturally infected. The extracts were applied for oral saw (intragastric), into the volume of 0.04 mL/g , with the employing of a dead and bend probe during three consecutive days. The fecal material, collected 24 hours after each application, performing a total of four fecal collection, have been softened previously, transferred about to sieve of network of 125 micrometers and tested under microscope stereoscope, with the objective of behave the identification and counting from the worms eliminated of the second to the fifth day of the experimental. Tukey-Kramer Multiple Comparisons Test was applied to compare the results. This approach intends to add new data on the helminthes parasites of laboratory mice. According to the analysis of the results it was observed that there were differences ($p<0.001$) in the %of elimination between 20% TM and 20% 20%UR (from 2.24 ± 3.33 to 2.92 ± 3.33), 20% TM and Nit (from 2.24 ± 3.33 to 64.0 ± 2.89), 20%TM and Meb (from 2.24 ± 3.33 to 100.0 ± 3.16), 20% UR and Nit (from 2.92 ± 3.16 to 64.0 ± 2.89) and ($p<0.01$) 20%UR and C (from 2.92 ± 3.16 to 1.56 ± 3.16). It was published that medicinal plants which were reported as useful in the treatment of diabetes the *S. lycocarpum* was the sixth most frequently mentioned. According to the results obtained in the present study, we can speculate that the anthelmintic effect of *Solanum lycocarpum* was noticed due to the concentration of steroidal alkaloid oligoglycosides and short-chain fatty acids. [Academia Arena, 2009;1(4):1-6]. ISSN 1553-992X.

Keywords: Anthelmintic; *Solanum lycocarpum; Aspiculuris tetraptera;* mouse

Introduction

The Brazilian flora is one the world richest sources of bioactive material due to its biodiversity. Several plants are currently used in Brazilian traditional medicine to treat diabetes. The starch obtained from the unripe fruits of *Solanum lycocarpum* St. Hill. (Solanaceae) has been widely used and commercialized as a hypoglycemic agent in Brazil. Recently studies carried out a chemical analysis of the starch and tried to correlate its supposed hypoglycemic activity with the polysaccharide content. However, these investigators did not conduct any experimental test to directly demonstrate the hypoglycemic effect attributed to the starch. As far as we know, no studies have evaluated the potential hypoglycemic effect of the starch of *S. lycocarpum* in experimental animals or the pattern of its use by a group of diabetic patients. *S. lycocarpum* is a plant which is shrubs ranging in height from 1.2 to 3 m. The fruit is yellow in color and resembles a medium sized tomato. Parts of the plant are poisonous if it gets in your system. When it is in bloom, it is medium blue. It blooms in the late winter, early spring, late fall, early winter, and mid winter. It is velvety or fuzzy. It needs water regularly. It is found in the Brazilian savannah but has been said to grow in San Antonio, Texas. *S. lycocarpum* is commonly used in Brazilian folk medicine. Solanaceae or lobeira is a plant used as a hypoglycemic agent. A study reported that the extract reduces glycemia in alloxan induced diabetic rats. It was reported that the potential of *S.lycocarpum* as antioxidant was capable reduce in 27% nitrate generation in diabetic animals. In literaturature has been demonstrated that *S.lycocarpum* is not ulcerogenic and restored haemoglobin and haematocrit to normal values in diabetic animals (Perez et al, 2006). Yoshikawa et al (2007) described that steroidal alkaloid oligoglycosides as solamargine, solasonine, and 12-hydroxysolasonine, inhibited the increase of rat serum glucose levels by suppressing the transfer of sucrose from the stomach to the small intestine.

It grows in wet, red clay. It needs water regularly. It doesn't need as much water in the winter because it needs full sunlight, and mild temperatures. They are edible by humans. This plant contains steroidal glycoalkaloids that can be transformed into an intermediate for steroidal drug production. In this way, it is very possible that these glycoalkaloids and its aglycone, once in the body by ingestion of *S. lycocarpum* fruits, may act by disrupting the endocrine system. Because its fruits may be consumed by pregnant animals in the fields, various studies determined the possible toxic effects of exposure to *S. lycocarpum* fruit from gestation. The unripe fruits contained 0.6% of solamargine and 0.9% of solasonine. It was related that *S. lycocarpum*, during gestation and the beginning of lactation reduces intrauterine growth. It is known that during adulthood, female offspring showed impaired sexual behavior and male offspring showed prominent degeneration of testis germinative cells, characterized by a reduced number of germ cells and vacuolation. It has been documented that the exposed offspring showed reduced hypothalamic norepinephrine (NOR), vanillylmandelic acid (VMA), 3-methoxy-4-hydrophenylglycol (MHPG) and homovanillic acid (HVA) levels, and reduced striatum NOR, HVA, VMA, MHPG, dopamine (DA), dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindolacetic acid (5-HIAA) levels. It is suggest that the fruit may act as an estrogen, with a long-term effect, impairing the receptive lordosis behavior of female offspring and promoting testis abnormalities in male offspring at adulthood. It appears to disrupt brain organization since important central monoamine level alterations were also related (Schwarz et al, 2005).

It was described by Vieira et al (2003) the anti-inflammatory effects of the crude ethanol extract and its alkaloid fraction from *S. lycocarpum* fruits. Due to the referred study the alkaloid fraction induced a dose-dependent reduction in ear edema formation and leukocyte migration, suggesting that *S. lycocarpum* fruits may contain steroidal alkaloids accounting for the anti-inflammatory effect of the crude ethanol extract.

Maruo et al (2003) demonstrated the embryotoxic effects of *S. lycocarpum* fruit ingestion during preimplantation and during organogenesis in rats. In this study few differences were observed in food and water consumption without biological importance. It was observed that the placental weight in the group that received the plant during the organogenesis period was decreased. An increase in sternebra abnormalities was observed in animals treated with the plant during organogenesis. Olfactory bulb hemorrhage was increased in the group that received the plant during preimplantation when compared to the control group. These results indicate that consumption of *S. lycocarpum* at 3% in diet during pregnancy cause slight toxicological effects. Chang et al (2002) evaluating the toxic effects of lobeira during the fetogenesis period, related that no clinical signs of maternal toxicity were observed. The placenta weights of the treated rats were lower than those of the control. lungs and kidneys of the fetuses treated with lobeira were also significantly reduced, suggesting a fetotoxic effect of this plant.

Rodents, as mice and rats are the most common laboratory animals used in research and testing. They are seldom investigated for autochthonous ecto- and endoparasites prior their utilization in the experiments. Pinworms commonly infecting laboratory rodents include mainly the mice pinworms *Syphacia obvelata* and *Aspiculuris tetraptera*, and in rats *Syphacia muris* (Matysiak et al, 2006). Fecal specimens obtained from rats and mice in general are infected with one or more helminth species. *Syphacia muris* and *Syphacia obvelata* are more frequently in rats, and *Aspiculuris tetraptera*, *S. obvelata* , in mice (Senlik et al, 2005).

Some plant extracts are efficient due to their anthelmintic activity. It was related that ethanolic and aqueous extracts obtained from nine plant species from seven families selected depending on their use in Turkish folk medicine, including *Citrillus lanatus* (Thunb.) *Matsum*. (seed), *Jasminum fruticans L*. (branches), *Juniperus drupacea Labill*. (fruits), *Juniperus nana L.* (fruit and leaves), *Juniperus oxcycedrus L* (fruit and leaves), *Mentha longifolia L.* (herba), *Pinus nigra ssp. pallasiana* (Lamb.) *Richt.* (fruits), *Plantago lanceolata L.* (leaves), and *Zea mays L.* (seed) were evaluated for their in vivo anthelmintic activity. Among the plant extracts studied, both ethanolic and aqueous extracts of *Jasminum fruticans*, *Mentha longifolia and Pinus nigra ssp. pallasiana*, the aqueous extracts of *Zea mays*, the ethanolic extracts of *Citrillus lanatus*, *Juniperus drupacea* (fruit), *Juniperus oxcycedrus* and *Plantago lanceolata* displayed significant anthelmintic activity against pinworms, *Syphacia obvelata* and *Aspiculuris tetraptera*, in mice. Rest of the extracts from plants did not show any remarkable anthelmintic activity (Kozan et al, 2006). Some plant extract may act differently due to its action against the parasite. In a study the anthelmintic activity of the extracts obtained from *Luxemburgia octandra* was evaluated naturally infected mice with *Aspiculuris tetraptera*. The leaves extracts were given to the animals during three days. The ethanolic and ethyl acetate extracts did not present the nematicide effect against *A. tetraptera* (Silva et al, 2005). In the

present study we evaluated the anthelmintic activity of *Solanum lycocarpum* extracts in a concentration of 20% in mice naturally infected *Aspiculuris tetraptera.*

Material and Method

Vegetal extracts: Dried leaves of units of had been used in the anthelmintic tests *Solanum lycocarpum* collected in the City of Três Marias, State of Minas Gerais and in the City of Seropédica, State of Rio de Janeiro. The botanical identification was carried through in the Department of Botany of the Rural Federal University of Rio de Janeiro, having been the exsiccates deposited under numbers RBR 28010 and RBR 14071. For the execution of the tests, the extracts had been gotten by infusion (tea), submitted to the filtration in nylon and the express concentrations in g/100 ml (p/v).

Animals and anthelmintic tests: For anthelmintc test have been used lots of albinos mice, male and females weighted in media of 25g and naturally infecting for *Aspiculuris tetraptera*, originated from Oswaldo Cruz Foundation – FIOCRUZ and held into the Institute of Biology from Rural Federal University from Rio de Janeiro. The animals have been held into bird cages individual of polypropylene (30x 20 x 13cm), it has at the bottom road of screen stark and stiff (network of 7x 7mm) upon a sheet of absorbent paper with the aim to facilitate the collection diary of excrement (Steward,1955, Amorim et al., 1987 e Amorim e Borba, 1990).

The extracts were applied for oral saw (intragastric), into the volume of 0.04mL/g , with the employing of a dead and bend probe during three consecutive days. The excrement, collected 24 hours after each application, performing a total of four fecal collection, have been softened previously, transferred about to tames of network of 125 micrometers (µm) and evaluated under microscope stereoscope, with the objective of behave the identification from the worm eliminated of the second to the fifth day of the experimental. Into the fifth and last days from the tests, the mice have been sacrificing for inhalation of vapors of ether ethyl, examining in the colon the number of the *A. tetraptera* remnants (Amorin et al., 1999). On the tests have been used the extracts of *Solanum lycocarpum* (leaves dried from Três Marias in the concentration of 20%) and (leaves dried from UFRRJ in the concentration of 20%). Additional lots of mice have been used with standard, they receiving doses of 20mg/kg/day of mebendazol and 100mg/kg/day of nitroscanato and they were submitted to the identical assessment anthelmintic description about to the animals treated with the plant extracts. A batch control, without a treatment served about to appraise the elimination spontaneous from the helminthes studied. The outcome antinematode also was denominated in terms percentile average of roundworm eliminated, considering the number of roundworm eliminated in the faecal material in relation to the total number. Statistical analysis were performed and Tukey-Kramer Multiple Comparisons Test was applied to compare the results.

Results

Table 1. Anthelmintic activity of the extracts obtained of *Solanum lycocarpum* in the elimination of *Aspiculuris tetraptera* in mice naturally infected.

Used Parts	Administration Form	Number of animals	Number of Helminthes			Elimination (%)
			Fecal	Necropsy	Exam	
Leaves Dried from Três Três Marias (TM)	20%	07	09		393	2.24
Leaves Dried from UFRRJ (UR)	20%	10	22		729	2.92
Nitroscanato (NIT)		12	499		282	64.0
Mebendazol (MEB)		10	324		0.0	100
Control (C)		10	45		2836	1.56

The extracts were applied for oral saw (intragastric), into the volume of 0.04mL/g , with the employing of a dead and bend probe during three consecutive days. The excrements, collected 24 hours after each application, performing a total of four fecal collection, have been softened previously, transferred about to tames of network of 125µm and evaluated under microscope stereoscope, with the objective of behave the identification of the worm eliminated of the second to the fifth day of the experimental. Tukey-Kramer Multiple Comparisons Test was applied to compare the results.

According to the analysis of the results it was observed that there were differences ($p<0.001$) in the %of elimination between 20%TM and 20% 20%UR (from 2.24 ± 3.33 to 2.92 ± 3.33), 20%TM and Nit (from 2.24 ± 3.33 to 64.0 ± 2.89), 20%TM and Meb (from 2.24 ± 3.33 to 100.0 ± 3.16), 20%UR and Nit (from 2.92 ± 3.16 to 64.0 ± 2.89) and ($p<0.01$) 20%UR and C (from 2.92 ± 3.16 to 1.56 ± 3.16).

Discussion

Although the objective of the present study was not to carry out a toxicological investigation of *S. lycocarpum* starch, we observed that the animals treated with the starch did not differ from those treated with the vehicle in terms of body weight changes during the experimental period. Many studies carried out on experimental animals have shown that steroidal alkaloids are generally toxic. Baker et al, (1989) have shown that Syrian hamsters orally treated with ground material obtained from Solanaceae species developed gastric and intestinal mucosal lesions. In addition, treatment of mice with steroidal alkaloids isolated from plants of this family also induced alterations of liver weight, arrhythmic beating in neonatal heart cells and neural-tube defects (Schwarz et al, 2005). Animal models have been exhaustively investigated regarding aspects related to their suitability for the development of experimental protocols under laboratory conditions. Nevertheless, in most of the adopted procedures, the prior detection of their ecto and endo parasites are generally overlooked related to the really effects of natural extracts in their biological cycle.

In the Brazilian cerate, a preparation obtained from the fruits of *Solanum lycocarpum* St.-Hill. (Solanaceae), popularly known as 'fruta-de-lobo' (wolf-fruit), have been widely employed for diabetes management, obesity and to decrease cholesterol levels. The medicinal preparation consists of the green fruits which are ground in aqueous solution and filtered. The white 'gum' deposited is decanted and slowly dried providing a powder which is commercialized in capsules with the name of 'polvilho-de-lobeira'. Through phytochemical analysis of this phytomedicine and the fruit of *S. lycocarpum* were found polysaccharides as the main component. Some polysaccharides slow gastric emptying and act on the endocrinous system affecting the liberation of gastrointestinal hormones, lowering blood glucose levels. According to Schwarz et al (2005) it is well known that this plant contain steroidal glycoalkaloids that can be transformed into an intermediate for steroidal drugs production, like oral contraceptives. In this way, it is very possible that these glycoalkaloids and its aglycone, once in the body by ingestion of *S. lycocarpum* fruits, may act disrupting to the endocrine system as well as it may probably affect the reproductive system of helminthes. The hypocholesterolemic activity could be due to the increased fecal bile acid excretion as well as to the action of the short-chain fatty acids, coming from fermentation, on the synthesis of delta-aminolevulinate and by the increase of the cholesterol 7-alpha-hydroxylase and 3-hydroxy-3-methylglutaryl CoA reductase synthesis (Dall and Lino, 2000).

Due to the effect related it may be possible that these fatty acids could act as an anthelmintic, although in he present study there was not observed differences between TM and UR extracts related to % of elimination in comparison one to another, although in comparison to the control group was evident a significative difference due to the UR group. Related to the obtained results due to the action of the UR extract it may be explained by their concentration as well as originated region which may explain the effect due to the biochemistry compounds in the equivalents proportions in spite of different conditions as soil composition, light and water availability.

The effect of UR extract may be support by possible modifications in ribosomal DNA spacer region suggesting that it could result in genetic and geographical variability as well as different bioactivity which may not be effective depend on the concentration of the extract (Arruda et al, 2003).

We can speculate that the other effect would be related to the low concentration of steroidal alkaloid oligoglycosides which in a optimal concentration may suppress the transfer of sucrose from the stomach to the small intestine which could diminish the support of glucose to helminthes together with its antioxidant effect which is capable of reducing the nitrate generation which can be used in the protein

synthesis as well as the possible inflamatory effect induced by the extract in the gastric and intestinal mucosal which could interfere in local homeostasis which is essentially to the develop of helminthes.

Conclusion

The results of the present study point to the need for a careful evaluation of the phytotherapic product in researching even when it may be widely used by the population. Based on the results we can suggested that the anthelmintic effect of *Solanum lycocarpum*, TM and UR extracts, is related to the possible concentration of steroidal alkaloid oligoglycosides as well as the short-chain fatty acids presents in the extract. The similar action of the extracts may be explained by adaptation mechanisms related to the genetic and geographical variability.

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