Original Article

Evaluation of the Acute Toxicity of the Hydroalcoholic Extract of *Solidago chilensis* Meyen (Arnica Do Campo) in Mice

Lyvia I.G. Paula-Freire[1], Elena L.A. Malpezzi-Marinho[2], Graziela R. Molska[1], Daniele O. Kohn[4], Luciana Correa[5] and Eduardo A.V. Marinho*[3]

[1]Faculdade de Ciências da Saúde, Universidade Braz Cubas, São Paulo, Brasil.
[2]Departamento de Ciências Biológicas, Universidade Estadual de Santa Cruz, Ilhéus, Bahia, Brasil
[3]Departamento de Ciências da Saúde, Universidade Estadual de Santa Cruz, Ilhéus, Bahia, Brasil
[4]Departamento de Psicobiologia, Universidade Federal de São Paulo, São Paulo, Brasil
[5]Departamento de Patologia Geral, Faculdade de Odontologia, Universidade de São Paulo, São Paulo, Brasil.

Address for Correspondence

BR-415, Rodovia Ilhéus- Itabuna, Km-16, Salobrinho, Ilhéus - Bahia, 45662-000, Brazil
E-mail: edumarinho@hotmail.com

ABSTRACT

Solidago chilensis is a plant with therapeutic properties that is widely used in folk Brazilian medicine. In this study, we evaluated the possible acute toxicity and determined the LD_{50} of the hydroalcoholic extract of *Solidago chilensis*. Swiss mice of both sexes received intraperitoneal injections of *Solidago chilensis* hydroalcoholic extract (ScHE) at the doses of 30, 100, 300, 500, 750 and 1000 mg/kg. Pharmacological screening, LD_{50} determination, food intake evaluation, body and target organ weight measurements and histopathological analyses were performed. The LD_{50} value was determined to be 512.5 mg/kg. Pharmacological screening revealed central nervous system depressant activity, as shown by ptosis and decreases in general activity and motor activity. Food intake and body weight were not affected by extract administration. Macroscopic analysis of organs indicated splenomegaly and changes in the color of the liver in animals treated with ScHE; both effects were dose dependent. Significant macroscopic changes in the kidneys were not observed. Histopathological analysis showed significant morphological alterations in the livers of animals treated with high doses of the extract. Only animals treated with 1000 mg/kg had hemorrhagic foci in the kidneys. Our findings indicate that the intraperitoneal injection of ScHE causes relevant toxicity, with a relatively high LD_{50} (512.5 mg/kg), and affects the histopathological parameters analyzed. Further toxicological tests should be performed using oral administration to compare the effects of different routes of administration and to simulate traditional use.

Keywords: *Solidago chilensis,* Toxicity, LD_{50}, Mice.

INTRODUCTION

The genus *Solidago* is considered as one of the largest genera of the Asteraceae family, comprising more than 120 species, most of which are found in North and South America. This genus comprises a great number of medicinal plants with therapeutic properties, including *S. virgaurea* and *S. gigantea*, the aerial parts of which are widely used as anti-inflammatory and diuretic agents. *Solidago chilensis* (*S. microglossa*), known in Brazil as *arnica-do-campo* or *arnica-brasileira*, is a plant found throughout the country, especially in the South and Southeast regions. This plant, high 80-120 cm, can be found in wastelands, roadsides, pastures, orchards and coffee plantations[1].

Ethnopharmacological data indicate that *S. chilensis* is broadly used in Brazil. At Rio de Janeiro, patches from leaves and stems are used on contusions[2], in many towns from Minas Gerais State *S. chilensis* is indicated to skin diseases, wound healings and boils, purulent infections, inflammation, edema, body pain, chill, rheumatism, diuretic, using leaves, flowers or the whole plant, by maceration, infusion, bath, decoction and tincture with topic and oral administration[3-5]. In Santa Catarina State it is used to skin lesions[6]. *S. chilensis* is in the Medicinal Plant Program of the Health Municipal Secretariat (Fitoviva) indicated to contusion, trauma and boils, as tincture and poultice from flowers and leaves[7]. Since 2009 *S. microglossa* is in the list of native medicinal plants used by Health Brazilian Program (SUS) and in the National List of Medicinal Plant (Renisus) that guides the scientific studies[8]. According to Corrêa *et al.*[9] it is toxic, should not be used in wounds, but without scientific evidences.

Several studies have demonstrated that both the extract and essential oil of *S. chilensis* contain compounds that have relevant biological activities. *S. chilensis* flower extracts have been shown to exhibit outstanding antioxidant activity and to be more effective than butylhydroxytoluene (an antioxidant used in food industry)[10]. Aqueous and hydroalcoholic extracts of *S. chilensis* exhibit topical and systemic anti-inflammatory activities similar to those of dexamethasone in rats and mice. This anti-inflammatory activity is not only due to the inhibition of pro-inflammatory mediators but also to reduce lymphocyte migration[11-13]. Other demonstrated activities include antifungal[14], anti-microbial[15], gastro-protective[16], hypo-glycemic[17], healing[18] and analgesic activities[19].

Despite the known pharmacological effects of *S. chilensis* and its traditional use as an alternative treatment for health problems, there have been few data about the possible toxic effects of this plant[16]. Therefore, we aimed to investigate the acute toxicity and to determine the LD_{50} of the hydroalcoholic extract of *Solidago chilensis* when administered intraperitoneally to mice. Possible histopathological alterations in target organs were also evaluated.

Material and Methods

Botanical material

Solidago chilensis was collected in the city of Guararema, São Paulo, Brazil. Botanical identification and authentication were performed by Dr. Lucia Rossi from the Instituto Botânico de São Paulo, São Paulo, Brazil, and one specimen was deposited in the herbarium under the number SP 397.047 (Figure 1).

Hydroalcoholic extract preparation and analysis

The aerial parts of the plant (flowers, leaves and stalks) were immersed in 93% ethanol (100 g/L) for one month. After this period, the hydroalcoholic extract was

filtered, concentrated using a rotary evaporator and then lyophilized. The dried material was referred to as the *Solidago chilensis* hydroalcoholic extract (ScHE) and was stored at 4°C until use. The dried extract was diluted in distilled water immediately prior to analysis. The *Solidago chilensis* hidroalcoolic extract was previously analyzed in its major constituents on work previously published by the group. Briefly, have been identified constituents as the 5-*O*-*E*-, 3,4-, 4,5-di-*OE*-caffeoylquinic acids and rutin[13].

Animals

Male and female Swiss albino mice (3 months old) obtained from the Braz Cubas University vivarium, were used. The animals were kept in a room with a controlled temperature of 21±1°C, a light/dark cycle of 12 hours (lights on at 7:00 A.M.) and free access to food and water. The study was conducted according to ethical principles[20] following institutional protocols and was approved by the local Committee for Ethical Surveillance in Animal Experimentation (SBCAL) for the proper use and care of experimental animals.

Acute toxicity study

Mice were equally separated into seven groups of four males and four females each. A range of doses of ScHE (30, 100, 300, 500, 750 and 1000 mg/kg) were administered once intraperitoneally. The vehicle control group received 0.3 mL of distillated water. The animals were observed for 14 days. The number of dead animals was recorded for each tested dose, and the lethal dose that killed 50% of animals (LD_{50}) was calculated according to the method of Carvalho *et al*[21].

$$LD_{50} = Df - Z\,(a.b) / n$$

Df = lowest dose able to kill all animals;

a = difference between 2 consecutive doses administered;
b = average number of animals killed between 2 consecutive doses;
n = number of mice by group;
z = sum of (ax b) / n.

Physiological and anatomopathological parameters

Pharmacological screening was also performed. The animals were observed continuously during the first hour after extract administration and at 2, 4, 12, 24 and 48 hours after extract administration[22]. The animals were also observed once per day for 14 days to check for residual effects. Food intake and body weight were analyzed daily. After 14 days, the animals were euthanized (cervical dislocation), and the liver, kidneys and spleen were removed, weighed and fixed in formalin (10%) for subsequent histological analysis. The organ weights were corrected based on the last body weight of the animals. Animals that died before the 14th day were autopsied, and the same parameters were analyzed.

Histopathology

Fixed material was embedded in paraffin, and sections (thickness: 5µm) were stained with hematoxylin/eosin. Histological analysis was performed using conventional microscopy by one pathologist who described the morphological alterations due to toxic effects.

Statistical analysis

Parametric tests were performed, taking into account variable characteristics. The data obtained from the food intake and body weight evaluations were analyzed by ANOVA with repeated measures, followed by Tukey's post hoc test. The organ weights were analyzed by one-way ANOVA, followed by Tukey's post hoc test. The values were presented as the mean ± standard error

of the mean (SE), and the significance level was taken as $p<0.05$.

RESULTS

LD_{50}

Table 1 shows the number of dead animals in each group. The LD_{50} of *S. chilensis* was 512.5 mg/kg body weight, as shown in Figure 2.

Initial pharmacological screening

Table 2 presents the most relevant signs observed in animals treated with different doses of ScHE by intraperitoneal injection. Mice exhibited a dose-dependent reduction in general activity relative to control animals for 48 hours after the administration of doses higher than 500 mg/kg, for 24 hours after the administration of the 300 mg/kg dose and for up to 4 hours after the administration of the 100 mg/kg dose. These results suggest that this extract has a depressant effect on the central nervous system. Reduced grip strength and ptosis were also observed 30 minutes after the administration of doses higher than 100 mg/kg. An increase in the respiratory rate was observed in mice treated with doses higher than 100 mg/kg after 30 minutes, and this increase was very apparent for doses of 500 and 1000 mg/kg. We also observed discrete cyanosis in the groups treated with doses of 100 and 300 mg/kg and intense cyanosis in the groups treated with doses of 500 and 1000 mg/kg one hour after ScHE administration. Piloerection also occurred for the 300, 500, 750 and 1000 mg/kg doses during the first 4 hours after extract administration. All animals treated with the plant extract at 1000 mg/kg died within two hours of administration. Animals treated with the 30 mg/kg dose did not exhibit any behavioral changes. It is important to note that there were no differences between males and females with respect to the analyzed parameters.

Physiological parameters

Figure 3 shows the body weight (A) and food intake (B) over the 14-day period after *S. chilensis* administration. No significant change was observed in the body weight after the administration of the plant extract. Food intake was significantly different only for the day after administration, during which animals that received 300 or 500 mg/kg consumed less food than control animals ($p<0.05$). Most of the animals treated with 750 mg/kg and all animals treated with 1000 mg/kg died within two hours of administration, providing insufficient data for this analysis.

Anatomopathological parameters

Macroscopic analysis of organs (liver, spleen and kidneys) was performed, and the color, texture and weight were evaluated. Figure 4A shows that the liver weight did not differ significantly among the control and ScHE groups. However, a significant difference was observed for color. Tissues exhibited spots and darkening in a dose-dependent manner (Figure 5). Only animals treated with the 500 mg/kg dose exhibited a significant increase in kidney weight (Figure 4B). The spleens from animals that received 300 or 500 mg/kg weighed more than spleens from control group animals (Figure 4C).

Histopathology

Liver slices from animals treated with 30 and 100 mg/kg ScHE exhibited normal architectures with preserved hepatic cords, but discrete vascular congestion and dilatation of the sinusoidal space were present (Figure 6, A and B). For the 300 mg/kg dose, intense chronic passive congestion and focal hepatocyte necrosis were observed without alterations of the hepatic lobule distribution (Figure 6C). Mice treated with 500 mg/kg exhibited steatohepatitis with evident vacuolar degeneration and focal necrosis (Figure 6D). For the 1000 mg/kg dose,

microscopic alterations due to toxicity were not observed, but generalized vascular congestion was present (most likely due the early death of these animals).

Only the animals treated with 1000 mg/kg exhibited intense vascular congestion in kidney cortex, with small hemorrhagic areas. The spleens did not exhibit morphological alterations that could indicate toxicity and did not differ from the control spleens at any of the tested doses.

DISCUSSION

Solidago chilensis is a plant that is widely used in folk medicine due to its well-known therapeutic properties[10,23,18,13]. However, there have been few toxicological studies performed on plants from this genus.

Our results suggest that acute treatment with *Solidago chilensis* hydro-alcoholic extract at a dose ranging from 30 to 500 mg/kg has little effect on the animal's body weight over a 14-day period (in the higher doses, the animals death and was not possible evaluate the weight). Food intake was decreased only on the day after extract administration for the 300 and 500 mg/kg groups. Despite this small change, animals treated with ScHE did not exhibit significant body weight changes.

The oral administration of aqueous extract of *S. chilensis* did not cause signs of toxicity in a previous study[16]. In the present study, however, the LD_{50} of the hydroalcoholic extract of this plant was determined to be 512.5 mg/kg, that this extract has high toxicity when administered intraperitoneally. Nevertheless, the doses used in the studies published in the literature were usually much lower than the LD_{50} that we determined in this study.

Based on the results obtained from the initial pharmacological screening, the *Solidago chilensis* extract exhibited a central effect at doses higher than 100 mg/kg. This effect manifested as ptosis and decreases in general activity and motor activity. These signs strongly suggest that the plant has a sedative effect when administered at high doses[22,24,25]. Symptoms such as cyanosis, respiratory rate alterations and piloerection were also observed for lethal doses before death, indicative of the pronounced toxic effect[22]. All animals that received the 1000 mg/kg dose died within two hours of administration, again indicating that this plant extract is toxic when administered intraperitoneally. It is important to note that the results obtained for the initial screening represent only a subjective impression of the drug's likely effect, but this screening is essential to determine the appropriate research direction.

The anatomopathological data indicate that there were significant changes in the livers and spleens of animals treated with ScHE and that these effects were dose dependent. The macroscopic observation of organs showed that livers from animals treated with *arnica do campo* exhibited darkened coloration, the extent of which depended on the administered dose, relative to the control animals. Additionally, animals treated with the plant extract exhibited splenomegaly (increases in spleen weight and volume). This response can occur due the presence of compounds with injurious activity. This activity can induce an inflammatory response, promoting organ enlargement (edema)[26].

The liver is the organ most affected by toxic substances due to its intense participation in biotransformation reactions[27]. It is known that splenomegaly can also occur due to liver failure, which promotes the congestion of the liver's blood vessels. This congestion is caused by the decrease in activity and by the increase in hydrostatic pressure. Organ enlargement causes blood to reflux into spleen through the splenic vein,

causing the spleen to swell[28]. No macroscopic alterations were observed in the kidneys.

The histopathological data show that the extract seems to affect the liver more intensely, and significant cellular alterations (such as hepatic steatosis, intense vacuolization and hepatocytes necrosis) were observed in mice treated with 300 mg/kg and higher doses of the extract. We also observed vessel congestion and hemorrhagic areas. Fat infiltration, blood congestion and hepatocyte necrosis are common signs of hepatitis and may cause death[29,30]. These data confirm the harmful effect of the extract on the liver that was observed in the macroscopic analyses.

Tests to evaluate the sub chronic toxicity (due to the repeated administration of lower doses) of ScHE are needed because the population uses this extract for extended periods of time[1]. Detailed analyses are also needed to determine which of the component compounds are toxic and if it is possible to remove them before use. Were commend that ScHE not be used by parenteral and enteral means.

CONCLUSION

In conclusion, *Solidago chilensis* hydroalcoholic extract exhibits significant toxicity when administered intraperitoneally, with a relatively high LD_{50}, and it affects anatomopathological and histopathological parameters. More studies should be performed because the important therapeutic effects of this plant.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Lucia Rossi from Instituto de Botânica de São Paulo, São Paulo, Brazil, for identifying the *Solidago chilensis* plant used to prepare the extract and to Dr. José Gregório Cabrera Gomes from Instituto de Ciências Biomédicas da Universidade de São Paulo, São Paulo, Brazil, for assistance in the preparation of the hydroalcoholic extract of this plant.

Authors' contributions

Lyvia I.G. Paula-Freire contributed in running the laboratory work, analysis of the data and drafted the paper. Elena L. A. Malpezzi-Marinho designed the study, contributed in collecting plant sample and identification, confection of herbarium, running the laboratory work, drafted the paper and supervised the laboratory work. Graziela R. Molska contributed in running the laboratory work. Daniele O. Kohn contributed to drafted of the paper. Luciana Corrêa contributed to anatomopathological and histopathological studies and drafted of the paper. Eduardo A.V. Marinho (PhD) designed the study, contributed in collecting plant sample and identification, confection of herbarium, running the laboratory work, analysis of the data and drafted the paper and supervised the laboratory work. All the authors have read the final manuscript and approved the submission.

REFERENCES

1. Lorenzi H, Matos FJA. Plantas medicinais no Brasil – nativas e exóticas. 1st ed., São Paulo: Instituto Plantarum de Estudos da Flora LTDA; 2002: 544p.
2. Posse JC. Medicinal plants used by SUS users in Paquetá and Santa Teresa neighborhoods. An ethnobotany approach. Rio de Janeiro. Dissertation. Post-Graduate Program in Pharmaceutical Sciences, Faculty of Pharmacy, Federal University of Rio de Janeiro, Rio de Janeiro (in Portuguese), 2007, 115p.
3. Calábria L, Cuba GT, Hwang SM, Marra JCF, Mendonça MF, Nascimento RC, Oliveira MR, Porto JPM, Santos DF, Silva BL, Soares TF, Xavier EM, Damasceno AA, Milani JF, Rezende CHA, Barbosa AAA, Canabrava HAN. Ethnobotanical and *Ethnopharmacological* survey of medicinal

plants in Indianopolis, Minas Gerais, Brazil. *Rev Bras Plantas Med* 2008, 10: 49-63.
4. Kffuri CW. Ethnobotany of medicinal plants in Senador Firmino (Minas Gerais State). Dissertation, Federal University of Viçosa, 2008, 88p.
5. Oliveira HB, Kffuri CW, Casali VWD. *Ethnopharmacological* study of medicinal plants used in Rosário da Limeira, Minas Gerais, Brazil. *Rev Bras Farmacogn* 2010, 20: 256-60.
6. Amorim CC. Ethnobotany of land medicine in the Coxilha Rica region, SC. *Rev Bras Agroecol* 2009, 4: 1596-99.
7. Jorge SSA. Plantas Medicinais: Coletânea de Saberes: www.agronomiaufs.com.br/index.php/downloads-e-videos/category/75-downloads (47825636 Plantas Medicinais. pdf) 2009.
8. Brasil.http://portal.saude.gov.br/portal/saude/area.cfm?id_area=1336; 2012.
9. Corrêa AD, Siqueira-Batista R, Quintas LEM. Plantas medicinais do cultivo à terapêutica, 7th ed., Petrópolis:Editora Vozes, 2008: 1247p.
10. Güntner C, Barra C, Cesio MV, Dellacassa E, Ferrando L, Ferreira F, Garcia C, González G, Heinzen H, Lloret A, Lorenzo D, Menendez P, Paz D, Soule S, Vasquez A, Moyna P. Antioxidant properties of *Solidago chilensis* L. flavonoids. *Acta Hortic* 1999, 501: 159-63.
11. Goulart S, Moritz MI, Lang KL, Liz R, Schenkel EP, Fröde TS. Anti-inflammatory evaluation of *Solidago chilensis* Meyen in a murine model of pleurisy. *J Ethnopharmacol* 2007, 113: 346-53.
12. Liz R, Vigil SV, Goulart S, Moritz MI, Schenkel EP, Fröde TS. The anti-inflammatory modulatory role of *Solidago chilensis* Meyen in the murine model of the air pouch. *J Pharm Pharmacol* 2008, 60: 515-21.
13. Tamura EK, Jimenez RS, Waismam K, Gobbo-Neto L, Lopes NP, Malpezzi-Marinho ELA, Marinho EA, Farsky SH. Inhibitory effects of *Solidago chilensis* Meyen hydroalcoholic extract on acute inflammation. *J Ethnopharmacol* 2009, 122: 478-85.
14. Duarte MC, Figueira GM, Sartoratto A, Rehder VL, Delarmelina C. Anti-Candida activity of Brazilian medicinal plants. *J Ethnopharmacol* 2005, 97: 305-11.
15. Morel AF, Dias GO, Porto C, Simionatto E, Stuker CZ, Dalcol II. Antimicrobial activity of extractives of *Solidago microglossa. Fitoterapia* 2006, 77: 453-55.
16. Bucciarelli A, Minetti A, Milczakowskyg C, Skliar M. Evaluation of gastroprotective activity and acute toxicity of *Solidago chilensis* Meyen (Asteraceae). *Pharm Biol* 2010, 48: 1025-30.
17. Melo AM, Bittencourt P, Nakutis FS, Silva AP, Cursino J, Santos GA, Ashino NG, Velloso LA, Torsoni AS, Torsoni MA. *Solidago chilensis* Meyen hydroalcoholic extract reduces JNK/IκB pathway activation and ameliorates insulin resistance in diet-induced obesity mice. *Exp Biol Med* 2011, 236: 1147-55.
18. Facury Neto MA, Fagundes DJ, Beletti ME, Novo NF, Juliano Y, Penha-Silva N. Systemic use of *Solidago microglossa* DC in the cicatrization of open cutaneous wounds in rats. *Braz J morphol Sci* 2004, 21: 207-10.
19. Silva AG, Sousa CP, Koehler J, Fontana J, Christo AG, Guedes-Bruni RR. Evaluation of an extract of Brazilian arnica (*Solidago chilensis* Meyen, Asteraceae) in treating lumbago. *Phytother Res* 2010, 24: 283-87.
20. Andersen ML, Tufik S. Animal models as ethical tools in biomedical research. 1st ed São Paulo: CLR Balieiro Editores, 2010: 563p.
21. Carvalho AAT, Sampaio MCC, Melo AFM, Souza IA, Higino JS. Estudos toxicológicos do extrato hidroalcoólico de *Psidium guajava* Linn. *Rev Bras Ciênc Saúde* 2002, 6: 43-50.
22. Carlini EA. "Screening" farmacológico de plantas brasileiras. *Rev Bras Biol* 1972, 32: 265-74.
23. Vila R, Mundina M, Tomi F, Furlán R, Zacchino S, Casanova J, Cañigueral S. Composition and antifungal activity of the essential oil of *Solidago chilensis*. *Planta Med* 2001, 68: 164-67.
24. Tabach R, Rodrigues E, Carlini EA. Preclinical toxicological assessment of a phytotherapeutic product--CPV (based on

dry extracts of *Crataegus oxyacantha* L., *Passiflora incarnata* L., and *Valeriana officinalis* L.). *Phytother Res* 2009, 23: 33-40.
25. Carlini EA. Plants and the central nervous system. *Pharmacol Biochem Behav* 2003, 75: 501-12.
26. Porfírio Z, Ribeiro MP, Estevam CS, Houly RLS, Sant'Ana AEG. Hepatosplenomegaly caused by an extract os cyanobacterium *Microcystis aeruginosa* bloom collected in the Manguaba lagoon, Alagoas, *Brazil. Rev Microbiol* 1999, 30: 278-85.
27. Matos LC, Martins B. Hepatites tóxicas: Revisão da literatura. *Med Interna* 2005, 12: 239-58.
28. Petroianu A. Drug-induced splenomegaly. *Acta Med Port* 2011, 4: 977-82.
29. Kim ST, Kim JD, Ahn SH, Ahn GS, Lee YI, Jeong YS. Hepatoprotective and antioxidant effects of *Alnus japonica* extracts on acetaminophen-induced hepatotoxicity in rats. *Phytother Res* 2004, 18: 971-75.
30. Begriche K, Massart J, Robin MA, Borgne-Sanchez A, Fromenty B. Drug-induced toxicity on mitochondria and lipid metabolism: mechanistic diversity and deleterious consequences for the liver. *J Hepatol*;2011,54:773-94.

Table 1. Number of deaths caused by the administration of *Solidago chilensis* hydroalcoholic extract at different doses

Groups	Number of Deaths
30 mg/kg	0/8
100 mg/kg	0/8
300 mg/kg	2/8
500 mg/kg	5/8
750 mg/kg	6/8
1000 mg/kg	8/8

Table 2. Summary of the observations during the initial pharmacological screening of mice treated with *Solidago chilensis* hydroalcoholic extract

Observed effects	Dose (mg/kg – I. p.)					
	30	100	300	500	750	1000
General activity		↓	↓	↓↓	↓↓↓	†
Ptosis			+	+	++	†
Grip strength			↓	↓	↓	†
Cyanosis			+	++	+++	†
Respiratory rate			+	++	+++	†
Piloerection			+	+	+	†
Writhing						†

(+) presence of effect; (↓) decrease; † death. Blanks represent no effect.

Figure 1. *Solidago chilensis* Meyen (photograph Eduardo A.V. Marinho)

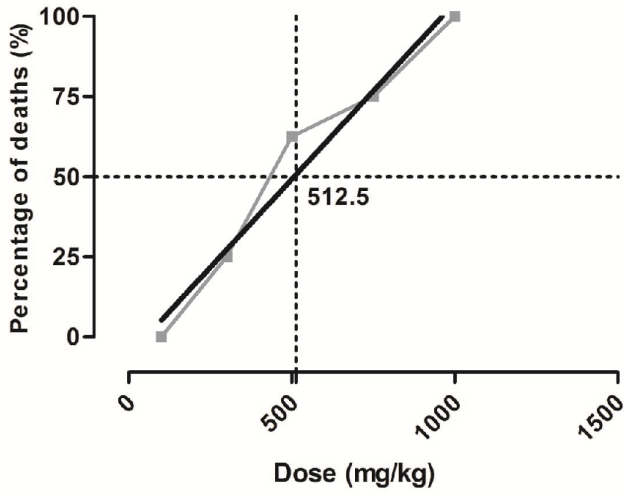

Figure 2. Graphic representation of the determination of the LD_{50} of the *Solidago chilensis* hydroalcoholic extract (512.5 mg/kg). The black line represents the linear regression of the data points in gray

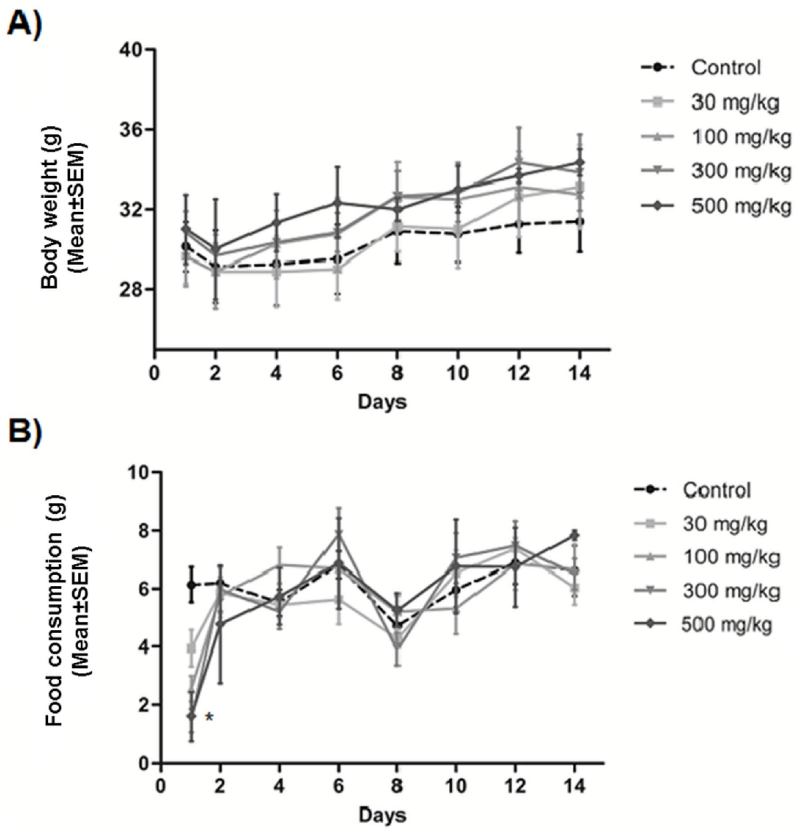

Figure 3. Body weight (A) and food intake (B) of the animals treated by intraperitoneal injection with increasing doses of *Solidago chilensis* hydroalcoholic extract. The values are expressed as the mean ± standard error of the mean. (*) Significantly different from the control group at the respective time (ANOVA with repeated measures followed by Tukey's *post hoc* test, $p<0.05$; n=8/group)

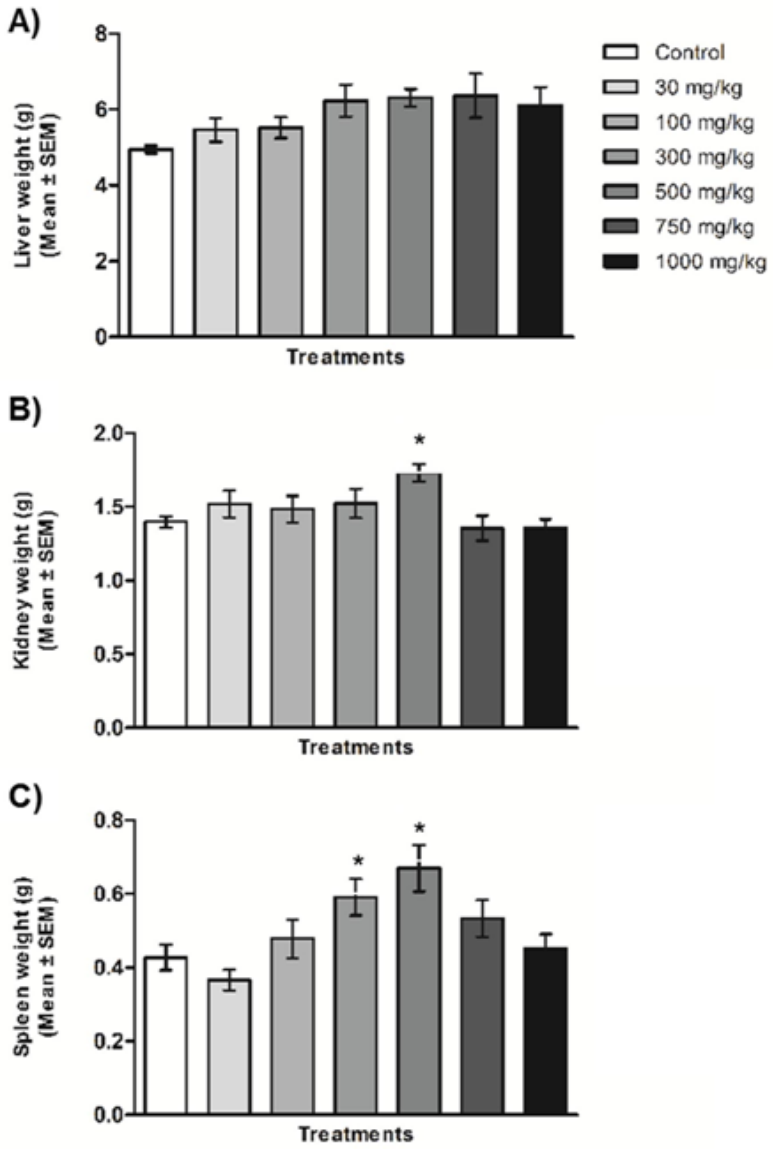

Figure 4. Weights of the liver (A), kidneys (B) and spleen (C), in grams, of the animals treated with water (control group) and *Solidago chilensis* hydroalcoholic extract at different doses. The values are expressed as the mean ± standard error of the mean. (*) Significantly different from the control group (ANOVA followed by Tukey's post hoc test, $p<0.05$, n=8/group)

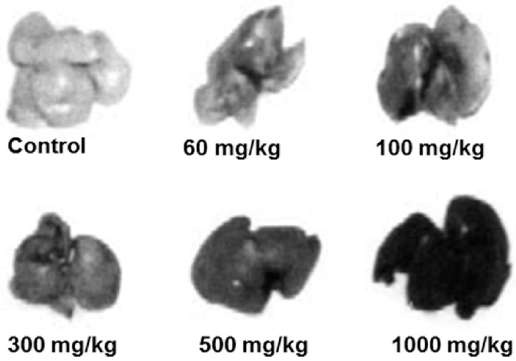

Figure 5. Photographs of the livers from animals treated with increasing doses of *Solidago chilensis* hydroalcoholic extract and from the control group

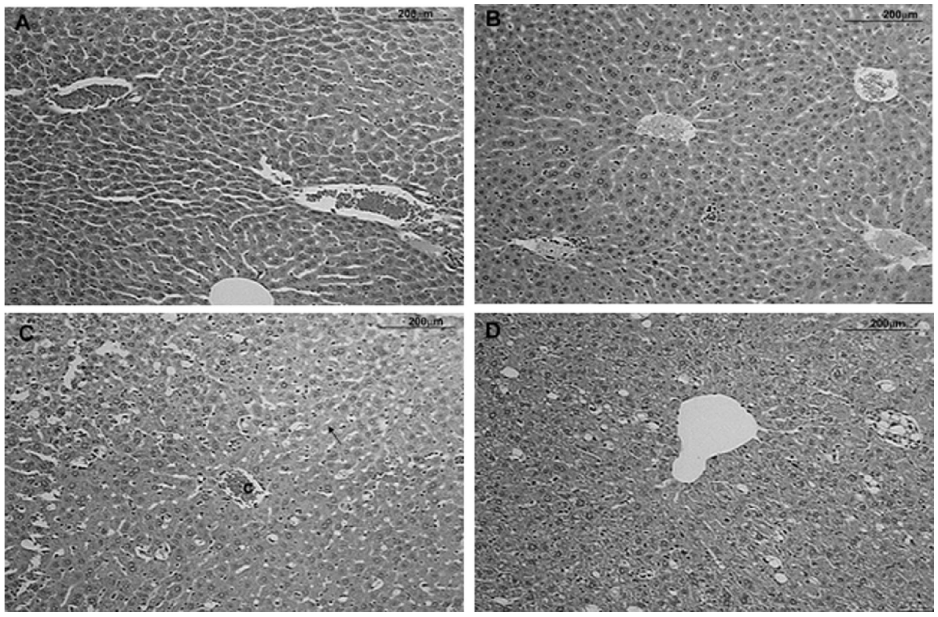

Figure 6. Liver slices from animals treated with the hydroalcoholic extract of *Solidago chilensis* at different doses: (A-30 mg/kg) Hepatocytes cords exhibited intercellular spaces that suggested dilatation of the sinusoidal space. The centrilobular veins exhibited slight passive congestion. (B–100 mg/kg) Hepatic lobules with stromal and parenchymal integrity. The centrilobular veins exhibited evident passive congestion. (C-300 mg/kg) Hepatic lobules with a centrilobular vein (c) exhibiting congestion and necrotic hepatocytes (arrow). (D-500 mg/kg) Steatohepatitis, with loss of the normal architecture of the hepatic lobules, vacuolar degeneration, and focal necrosis (HE, 100X)