

Acute and subacute toxicity of the hydroalcoholic extract from *Wedelia paludosa* (*Acmela brasiliensis*) (Asteraceae) in mice

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Abstract PURPOSE. The present study was carried out to evaluate acute and subacute toxicity of a hydroalcoholic extract from aerial parts of *Wedelia paludosa* (Asteraceae). **METHODS.** Toxicity of *W. paludosa* was evaluated in Swiss mice after ingestions of the extract during one day (acute model) and during 15 days (subacute model). **RESULTS.** The results showed that the LD₅₀ of the extract is higher than 4000 mg/kg and the subacute treatment did not show any change in corporal weight and hematological parameters. However, a change in liver weight but not in hepatic enzymes was observed. This suggests that the liver function is not altered by *Wedelia paludosa* in this study. Some changes in the creatinine content were observed, but could not be related with the extract dose. **CONCLUSIONS.** The results suggest that the plant seems to be destituted of toxic effects in mice.

INTRODUCTION

Wedelia paludosa DC, recently reclassified as *Acmela brasiliensis* (Asteraceae), is an ornamental plant, being widely used in natural medicine in South Brazil. This plant is used for the treatment of several ailments, including respiratory infections and pain (0, 0). Our research group has previously investigated the phytochemical and pharmacological properties of this plant. In this context, its analgesic (0, 0), antimicrobial and antidiabetic effects (0), were described and related with the presence of terpenes and flavonoids, including kaurenoic acid and luteolin (0, 0, 0). Other authors showed that kaurane diterpenes from *W. paludosa* display

trypanosomicidal activity (0) and smooth muscle relaxant effect (0). Due to the widespread use of this plant by the rural communities to treat several diseases, the objective of the present study was to obtain data on the safety of the crude extract. The acute and subacute oral toxicity of the hydroalcoholic extract from aerial parts of this plant in mice was assessed. The changes in selected biochemical and hematological parameters were also determined.

METHODOLOGY

Plant material

The aerial parts of *Wedelia paludosa* DC were collected next to NIQFAR/UNIVALI, in the town of Itajaí, in the State of Santa Catarina. The plant material was authenticated by Dr. Ademir Reis (Department of Botany, UFSC, Florianópolis) and a voucher specimen was deposited at the Barbosa Rodrigues Herbarium (Itajaí), under number VC Filho 002. The aerial parts of the plant were air-dried, cut into small pieces and macerated with 50 % ethanol (w/w) at room temperature for 15 days. After filtration, the solvent was removed under reduced pressure and the hydroalcoholic extract was then obtained.

Animals

Acute toxicity

The toxicity study was carried out using female and male Swiss mice (25-35 g). Animals were kept in a temperature-controlled environment (23 ± 2°C) with a 12 h light-dark cycle and food and water were freely available. Ethics Committee of UNIVALI approved the protocol for these experiments under number 314/2004. The animals were divided into one control group and five treated groups, each group consisting of ten animals. The control group received saline and each treated group received the hydroalcoholic extract in a dose of 100, 500, 1000, 2000 and 4000 mg/kg by gavage. These doses were chosen because they were 10-100 times higher than effective doses in other studies. The animals were observed continuously for 3 h, and then they were observed each hour during 24 h after administering the extract to observe any changes in general behavior or other physiological activities. At the end of the experiment animals were sacrificed by cervical displacement.

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Subacute toxicity

Female Swiss mice, 25-35 g, were kept in a temperature-controlled environment ($23 \pm 2^\circ\text{C}$) with a 12 h light-dark cycle. Food and water were freely available and were recorded each 3 days. The animals were divided into one control group and four treated groups (500, 1000, 2000 and 4000 mg/kg), each group consisting of ten animals. The control group received saline and each treated group received the hydroalcoholic extract by gavage for 15 days (once a day). The animals were weighed each 3 days. At the end of the experiment, blood was collected from the orbital sinus under ether anesthesia for biochemical and hematological analysis. After the blood collection, the animals were sacrificed by cervical displacement and selected organs (liver, heart, spleen, left kidney and left lung) were removed for macroscopic analysis. The biochemical parameters evaluated included creatinine, calcium, inorganic phosphorus, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase and serum alkaline phosphatase and were assessed using commercial kits. The hematology parameters were determined for the control and 1000 mg/kg groups and included red blood-cell count, hematocrit and leukocyte count.

Statistical analysis

The results are presented as mean \pm s.d. and the statistical significance between the groups was analyzed by means of an analysis of variance followed by Dunnett's multiple comparison test. P values less than 0.05 were considered as significant.

RESULTS AND DISCUSSION

The use of herbal preparations as in the treatment of diseases is very common in the rural communities of Brazil. *Wedelia paludosa* is frequently used for the treatment of infections, pathologies of the respiratory tract, fever and others diseases involving inflammation and pain (0, 0). The importance of this plant in folk medicine as well as its promising pharmacological properties verified in our laboratories (0, 0, 0, 0, 0), make studies about its toxicity very important.

Oral administration of the hydroalcoholic extract of *W. paludosa* in doses from 100 to 4000 mg/kg did not produce significant changes in behavior, breathing, cutaneous effects, sensory nervous system responses, and gastrointestinal effects in male and female mice. These effects are

observed during the experimental period (24 h). During 24 h of the experiment, no deaths occurred in any of the groups. These results showed that in single dose, there are no adverse effects of *W. paludosa*, indicating that the medium lethal dose (LD_{50}) is higher than 4000 mg/kg for male and female mice.

The results showed that a hydroalcoholic extract from *Wedelia paludosa* is safe in oral administration in rodents. The doses used in this study were 10 - 100 times higher than those used in other experimental pharmacological studies, such as: decrease of blood glucose levels in alloxan-induced diabetic rats (300 mg/kg) (0); inhibition of pain caused by intraperitoneal administration of extract on writhing test in mice (1mg/kg of roots extract and 3 mg/kg of leaves and stem extracts (0).

The treatment with the extract did not decrease the water and food consumption (data not show). The body weight (table 1) of the animals treated with hydroalcoholic extract once a day during 15 days (subacute treatment) did not show any significant change when compared with the control group, although had a tendency to decrease body weight (2000 and 4000 mg/kg). This decrease can be associate with the decrease of liver weight at the doses of 2000 and 4000 mg/kg in comparison with the control group without any concomitant alteration in the activity of alanine aminotransferase, aspartate aminotransferase, and gama-glutamyl transferase. Estimation of the serum activity gamma-glutamyl transferase, alanine aminotransferase and aspartate aminotransferase is one of the most widely used means of measuring hepatocellular injury (0). The macroscopic analysis of the target organs of the treated animals (liver, lung, heart, spleen and left kidney) did not show significant changes in color and texture when compared with the control group. The results of organs weight are summarized in table 1.

During the experimental period, there were no treatment-related effects on the hematological parameters evaluated (table 2). On the other hand, alterations in the serum concentration of creatinine were observed (table 3). As the macroscopic appearance and weight of the kidney was not altered, hence, the possibility of renal injuries could not be confirmed.

Table 1. Effect of oral administration of *W. paludosa* extract on body and organs weight.

Dose (mg/kg):	Control	500	1000	2000	4000
Body (g)	34.1 ± 3.17	34.7 ± 1.90	36.0 ± 3.77	32.0 ± 3.8	33.0 ± 2.77
Liver (g)	1.559 ± 0.200	1.415 ± 0.140	1.468 ± 0.225	1.3 ± 0.177*	1.12 ± 0.176**
Heart (g)	0.144 ± 0.018	0.151 ± 0.017	0.163 ± 0.037	0.155 ± 0.028	0.12 ± 0.028
Left lung (g)	0.213 ± 0.037	0.191 ± 0.016	0.235 ± 0.040	0.242 ± 0.064	0.18 ± 0.040
Spleen (g)	0.186 ± 0.092	0.156 ± 0.031	0.165 ± 0.051	0.126 ± 0.013	0.14 ± 0.0296
Left kidney (g)	0.165 ± 0.021	0.157 ± 0.017	0.175 ± 0.025	0.171 ± 0.027	0.14 ± 0.0237

Mean values of 10 animals ± S.D. * $p < 0.05$; ** $p < 0.01$ vs. control group (Dunnett's test). Control group received saline. No significant difference was observed in any parameter, except in liver (2000 and 4000 mg/kg).

Table 2. Hematological parameters after 15 days treatment with the *W. paludosa* extract.

Parameter	Control	1000 mg/kg	4000 mg/kg
Red blood cell (mm ³)	9.043 ± 0.370	8.095 ± 0.451	8.81 ± 0.129
Hematocrit (%)	46.1 ± 4.63	44.1 ± 1.524	47.049 ± 2.63
Leukocyte (x10 ⁶ /mL)	7.550 ± 2.192	8.730 ± 2.493	7.14 ± 1.689

Values are mean of 10 animals ± S.D. (Dunnett's test). No significant difference was observed in any parameter.

Table 3. Effect of treatment with *W. paludosa* extract on biochemical parameters.

Dose (mg/kg)	Control (n=9)	500 (n=10)	1000 (n=9)	2000 (n=8)	4000 (n=7)
Creatinine (mg/dL)	0.259 ± 0.050	0.285 ± 0.04	0.159 ± 0.04**	0.215 ± 0.06	0.248 ± 0.025
Calcium (mg/dL)	7.489 ± 0.528	7.976 ± 0.91	7.754 ± 0.322	8.154 ± 0.382	8.317 ± 0.807
Inorganic phosphorus (mg/dL)	6.650 ± 0.547	6.210 ± 0.62	6.770 ± 0.641	6.410 ± 1.162	6.836 ± 0.671
ALT (U/L)	111.9 ± 21.20	113.9 ± 10.5	91.99 ± 19.89	98.29 ± 31.77	90.51 ± 15.25
AST (U/L)	54.65 ± 10.15	51.57 ± 8.61	52.38 ± 8.072	48.88 ± 8.059	53.67 ± 12.44
Gamma-glutamyl transferase (U/L)	2.133 ± 1.920	2.780 ± 1.83	2.03 ± 1.481	2.401 ± 2.81	3.42 ± 1.616

Values are mean ± S.D. ** $p < 0.01$ vs. control group (Dunnett's test).

ALT – alanine aminotransferase

AST – aspartate aminotransferase

Administration of hydroalcoholic extract of *W. paludosa* in mice not induced any changes in these enzymes, although the liver weight decreased. This observation could indicate that liver function is preserved by oral administration of *W. paludosa*. It's important to mention that studies on other species of the genus *Wedelia* showed that: i) *W. calendulacea* has protective activity against liver injury *in vivo* (0); ii) *W. glauca* caused liver necrosis in rats (0), sheep and cattle (0); iii) *W. chinensis* has an hepatoprotective effect against liver injury (0).

In summary, our study demonstrated that *W. paludosa* seems to be destitute of toxic effects, which could be compromise the medicinal use of this plant in folk medicine. However, further studies are necessary, such as histological and morphological experiments, to confirm this evidence.

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