# TOXICOLOGICAL EVALUATION OF AN INFUSION OF *Bidens pilosa*

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## Summary

Introduction: *Bidens pilosa* (romerillo) is a widely used medicinal plant for the treatment of many diseases and it has been scientifically proved a lot of pharmacological actions. The objective of this paper is to determine if its potential toxicity after the oral administration of an infusion of this plant in an single and repeated dose (28 days) using in both cases limit doses and to assess if this preparation was a dermal irritant. Materials and Methods: acute toxicity was evaluated in 2 groups of 10 S/D rats each (5 males and 5 females), group 1 was treated and 2 was negative control. In repeated dose toxicity were conformed 3 groups of 20 animals each (10 males and 10 females), group 1 was treated with the infusion, group 2 treated with distilled water and number 3 was sentinel. Products were administered orally 6 days a week. Passed 28 days animals (groups 1 and 2) were sacrificed and group 3 was done so 30 days after. Haematological and biochemical variables were measured and indicated organs were histopathologicaly evaluated. Dermal irritancy was carried out using 3 rabbits for each preparation. Results: toxicological effects were absent in the single dose study and repeated dose study behave in the same way. There were no oedema or erythema in dermal studies. Conclusions: it is proved that the infusion of *B. pilosa* is non toxic for single and repeated dose also for dermal structures.

Keywords: *Bidens pilosa*, toxicology dose repeatedly, CTA, skin irritability.

## Introduction

*Bidens pilosa* (romerillo) is a medicinal plant very used popularly which are attributed numerous pharmacological actions (1). The main active principle is the hepta-2,4,6 triino-7-phenilo (phenilheptatrino) (2). Other active principles which pharmacological properties are the tannins (3), and flavonoides among them quercetin and its glycosides are found (4).

Many preclinical trials have been carried out to verify the pharmacological effectiveness of the *Bidens pilosa*, which have shown their antioxidant and immunomodulatory (5), diuretic (6) (7), antimalarial (8,9), anticancer and antipyretic (10), hypotensive (11), antileukemic (12), anti-ulcerogenic (13) effects. Nevertheless the toxicological evaluation of this medicinal plant has not been sufficiently studied, so the objective of this work was to carry out the toxicological evaluation of an infusion of this plant.

## Methods

The infusion was prepared with the dry ground material utilizing 40 g in 250 mL. of seething water, resting it for 10 minutes before filtering. The infusion was rotoevaporated until obtaining a total solids concentration of 200 mg/ mL for the toxicological study and of 100 mg/ mL for the repeated dose toxicity study. For dermal irritation study the infusion was used without evaporative procedures.

In the acute toxicology the biological model utilized was rats Sprague Dawley, by the method of the classes by the guideline No. 423 OCDE (14). Two groups were utilized, the group 1 received 2,000 mg / Kg BW and the group 2 functioned as negative control. Each group had 10 animals (5 females and 5 males). For the oral repeated dose toxicity study was carried out by the guideline No. 408 of the OCDE (15). The biological model utilized was rats Sprague Dawley, that had an initial weight understood between 150 and 160 grams. There were conformed 3 groups of 20 rats each one in a relation of sex of 1: 1. The group 1 was the treated group that received the infusion in a dosage of 1000 mg/Kg, the group 2 was the control that received water and the group 3, sentinels, that was left 30 days later to the last dosage before being sacrificed. The haematological and biochemical variables were determined, thus the organs were evaluated macro and microscopically. For dermal irritation studies 6 rabbits were utilized and applied 0,5 mL of the gel of the cream in an area of 6 $cm^2$ of skin, neighbour zones were taken as control zones.

## Results

**Study of acute toxicity (limit dose) of Bidens pilosa L.** (14)

Non toxic symptoms in the treated animals were observed, neither the first day of the test nor during the 14 remaining days. Clinical evaluation was normal, the consumption of food and water were also normal, no death occurred. The weight behaved in an adequate form for the sex and the increment of the corporal weight was also of an adequate form.

**Repeated dose toxicity study (28 days) of Bidens pilosa L.** (15)

The administration of the infusion did not produce signs or symptoms of apparent toxicity during the time that the experiment lasted and in sentinel group during the post administration month. The weight behaviour can be observed in the Table 1.

**Table 1. Rat's weights in the repeated dose toxicity study (28 days)**

| T Groups | N | Initial weight (g) | Final weight (g) | Weight increments (g) |
|---|---|---|---|---|
| Treated Males | 8 | 152,25 ± 36,88 | 313,37 ± 12,73 | 161,13 ± 38,47 |
| Treated Females | 9 | 154,00 ± 4,12 | 261,78 ± 15,86[(a)] | 116,77 ± 15,63[(a)] |
| Control Males | 10 | 161,30 ± 10,79 | 308,40 ± 19,70 | 147,10 ± 22,23 |
| Control Females | 10 | 149,90 ± 4,70 | 241,50 ± 12,14 | 91,60 ± 12,69 |
| Sentinel Males | 8 | 160,50 ± 8,81 | 385,88 ± 38,26 | 225,37 ± 35,86 |
| Sentinel Females | 9 | 148,44 ± 4,71 | 255,44 ± 5,02 | 107,0 ± 7,87 |

a) Significant difference between the treated group and control ($p < 0,05$).

The haematological evaluations carried out threw normal levels of the parameters evaluated, being within the physiological rank.

Table 2 reflects the result of the biochemical variables studied

**Table 2. Test Biochemical variables**

| Groups | N | Glucose mmol/L | Creatinin µmol/L | Proteíns g/L | Albumin g/L | TGP U / L | Iron µ mol / L |
|---|---|---|---|---|---|---|---|
| **Group 1 Treated** | **17** | 8,39 ± 1,5 | 21,09 ± 6,6 | 56,91 ± 5,4 | 38,73 ± 3,8 | 45,93 ± 6,7 | 42,4± 13,8 |
| **Group 2 Control** | **20** | 7,82 ± 0,7 | 23,99 ± 4,3 | 56,29 ± 4,9 | 37,75 ± 3,2 | 49,22 ± 8,6 | 41,3± 12,8 |
| **Group 3 Sentinel** | **17** | 6,45 ± 1,3 (a,b) | 35,06 ± 8,7 (a) | 65,85 ± 8,6 (a,b) | 43,60 ± 4, 6 (a,b) | 40,54 ± 8,8(b) | 52,3± 13,4 (a,b) |
| **Normal Rank** | - | **4,71– 7,33** | **30- 120** | **63 -86** | **34 - 48** | **17- 50** | **6 - 55** |

Mean (p <0,05): a) Control group and sentinel; b) treated Group and the sentinel; c) Group control and sentinel; d) treated Group and sentinel

**Study of the skin irritability of the gel and the cream of *Bidens pilosa*.**

The calculated Primary Irritation Index of both formulations prepeared from *Bidens pilosa* was equal to zero, so both preparations pass the test.

## Discussion

**Study of acute toxicity (limit dose) of *Bidens pilosa L.*** (14)
As some authors has reported (1,4), *B. pilosa* is a widely used plant with no previous reports of toxicity confirming the results of this investigations and allowing to clasiffy this plant as Non toxic under our experimental conditions.

**Repeated dose toxicity study (28 days) of *Bidens pilosa L.*** (15)
Even there were not found any previous report of repeated dose toxicty study of *Bidens pilosa* thus we analyzed every aspect, in first place, body weight behaved of an adequate form being superior in the groups that received the test substance, what speaks of its correct assimilation indicating the absence of toxic effects associated to treatment.

Haematological parameters showed a very highly significant increment of haemoglobin in the sentinel group when is compared with the control and treated group, what indicates that the animals that received the treatment raised the synthesis of haemoglobin in the post administration period.

Haemochemical data showed that glucose is slightly elevated in the group of processing and in the control staying in physiological rank for this variable. It calls the attention that the application of the infusion to this dose did not produce the decrease of glucose in blood as was previously reported (16). The increment in both groups induces us to think that this elevation can be owed to stress that produced the daily oral administration besides these levels descends significantly to the normal values in the sentinel group. The creatinin is under the physiological rank in the treated group and in the control. In the sentinel group this variable reaches the physiological rank and presents very highly significant difference with control and treated groups. The total proteins and the albumin in the treated and control groups are below the normal range, and they do not present significant differences, both variables recover their normal levels after 1 month suspended the supply of infusion and the group sentinel shows a very highly significant increment in relation to the treated and control groups. The urea, cholesterol, transaminases TGO and TGP, calcium and iron values were in all the groups in the physiological rank and besides there were no significant differences among them. A significant decrease of TGO in sentinel group is produced and a significant increment of the content of iron with regard to the groups control and treated.

In the pathological study, no macroscopic alterations were found that could be attributed to the infusion. The microscopic study did neither throw tissue damage in none of the structures analyzed

**Study of the skin irritability of the gel and the cream of *Bidens pilosa*.**
Dermal toxicity studies results are according to preliminary informations (1,4) and are of a great importance keeping in mind the UV protective and antioxidant (5) effect of this infusion, so we can say that both can be used with cosmetic puerpuse to protect the skin.

## Conclusions

The infusion of *Bidens pilosa* does not produce acute oral toxicity at a limit dose of 2000 mg/ Kg, does neither produce toxicity after the application of repeated doses of 1000 mg/ Kg during 28 days, being produced some beneficial changes on haematological and biochemical variables in the sentinel group. So much the gel as the cream prepared from the infusion of *Bidens pilosa* did not produce dermal irritation.

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