

ACUTE TOXICITY EVALUATION OF THE ETHANOLIC EXTRACT FROM LEAVES AND FLOWERS OF *CHROMOLAENA PERGLABRA* (B. L. ROBINSON) KING & H. ROB.

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Abstract

At present, there is a great variety of plant species widely distributed in different geographical regions worldwide, with phytopharmacological potential; however, its indiscriminate use has limited access to vegetal resources by the population in general and the scientific community. Additionally, the lack of toxicological studies that provide knowledge on the safety of their use makes the problem more critical. According to traditional knowledge, the species that belong to the genus *Chromolaena*, have a high pharmacological potential, but currently, there are no studies that validate traditional use and safety. Therefore, the purpose of this investigation was to evaluate the acute toxicity of the leaf and flower extract of *Chromolaena perglabra* (*C. perglabra*) in mice. To develop this aim, 25 female ICR-CD1 mice were divided into two groups with three and two subgroups respectively, to which they were administered intraperitoneally 125, 250, 500, 1000 and 2000 mg / kg of the ethanolic extract of leaves and flowers of *C. perglabra*; another 5 mice were administered the vehicle (control group); 72 hours after inoculation, they were sacrificed for histopathological study (macroscopic and microscopic). An increase in size was observed with mild lymphoid hyperplasia in the spleen and toxic hepatitis in all the animals, the rest of the organs analyzed were in normal conditions.

Keywords: Acute toxicity, *Chromolaena perglabra*, Asteraceae, medicinal plants.

Introduction

The use of medicinal plants dates back to ancient civilizations around the world. As a result, it is well known that commercially they are found in the market and in the free access of the population, drugs with uses described and transmitted from ancestral generations, however, despite of scientific advances, there are no studies that evaluate the safety of the so-called "herbal medicine" for its use in humans [1, 2].

The foregoing demonstrates that the search for control of the disease with drugs provided by nature, becomes almost instinctive, forcing the research community to generate scientific bases that allow the use of medicinal plants or their by-products, in the treatment and / or prevention of some groups of pathologies, highlighting anti-inflammatory, antifertility, immunomodulatory, antidiabetic, antimicrobial, antihypertensive, antipsychotic, among others. Some of these, in contrast to conventional pharmacology, have achieved a reduction of the effective dose or definitive suspension of the synthetic agent by remission of the disease, which could possibly avoid side effects [1, 3-11].

In Colombia in particular, there is a great variety of plant species widely distributed in different geographical regions and it is estimated that around 6 thousand of them have medicinal properties and have contributed to the development of phytotherapy in the country, however, for more than a decade, the need for phytochemical, pharmacological and toxicological evaluation is exposed [12, 13].

The information in the literature is not extensive about the Asteraceae family. Different properties have been attributed to this botanical family, among which are antioxidant, due to its flavonoid content, antimicrobial, anti-inflammatory, explained by the inhibition of molecules such as prostaglandins, nitric oxide and tumor necrosis factor and hypouricemic, promoting the excretion of uric acid at the intestinal [14-17]. However, these studies do not describe the side effects of the evaluated species, such as ovarian destruction, serum decrease of hormones related to female fertility, changes in the placental structure of pregnant mice as well as the decrease in the survival of the product after birth [18, 19].

Acute toxicity studies are few and focus only on a small number of plant species [20-22].

Specifically on the genus *Chromolaena*, some experts have achieved chemical characterizations that allow an approximation to their biological and pharmacological potential, however, of all existing species, there are few that have serious research, not only in Colombia but in general at the worldwide, where only *Chromolaena odorata* has acute toxicity studies [23, 24]. Therefore, the purpose of this investigation was to evaluate the acute toxicity of the leaf and flower extract of *Chromolaena perglabra* (B.L. Robinson) King & H. Rob in female ICR-CD1 mice.

Materials and Methods

Plant Material

The plant material of *C. perglabra* was collected in Tinjacá (Boyacá, Colombia; 2175 m.a.s.l, 17°C). Leaves and flowers were identified at the National Herbarium of Colombia (voucher number: COL407570).

Extraction

The leaves and flowers of *C. perglabra* were dried at room temperature. Subsequently, the dried plant material was ground, obtaining 500 g. The dry and milled material was extracted with ethanol (EtOH) in a soxhlet equipment at 50°C, for 5 days; Subsequently, by evaporation under vacuum, it was concentrated and the total ethanolic extract (ET) was obtained.

Preliminary phytochemical tests

A qualitative phytochemical evaluation was carried out in order to identify the presence of different secondary metabolites such as flavonoids (Shinoda reaction and Zn/HCl assay), steroids (Liebermann-Burchard test and Hager-Salkowski reaction) and saponins (test of foam).

Animals

30 female ICR-CD1 mice of 8 weeks of age, nulliparous, non-pregnant, with 20 g weight, were obtained from National Institute of Health (INS-Colombia). The animals were housed in 3 independent conventional cages, at 20 °C (±2), relative humidity of 75% with cycles of 12 light / dark hours, balanced diet (Rodentina) and water ad libitum.

Acute toxicity

For the acute toxicity test, the mice were divided into 3 groups, the first (G1) conformed by 15 mice

and subdivided into 3 subgroups (G1a n = 5, G1b n = 5 and G1c n = 5), the second (G2) by 10 mice and 2 subgroups (G2a n = 5 and G2b n = 5) and the third (G3) corresponded to the control group (n = 5). Each of the subgroups of mice of G1 and G2 were administered 0.5 ml intraperitoneally (IP) of a dilution of the extract of *C. perglabra* at different concentrations in sterile water (Table 1). Group 3 only received distilled water at the same volume and by the same route of administration (Figure 1). In this study, the OECD guidelines were followed (25). During the experiment, the general activity of the animals: irritability, response to touch, contortions, general posture, body tone, pressure force, lacrimation, piloerection, diuresis and defecation were daily evaluated.

Histopathological study

After 72h the IP injection, the animals of G1 and G2 were sacrificed by cervical dislocation to proceed to sampling of vital organs (liver, spleen, stomach and kidney). The tissues were preserved in a formaldehyde solution, fixed with liquid paraffin and stained with hematoxylin-eosin; cuttings of 3-4 microns were made. Subsequently, the samples were sent to the pathology laboratory for analysis (Figure 1).

Ethical aspects

The ethics committee of University to ensure the welfare of animals evaluated the project.

Statistical analysis

One-way ANOVA analysis was performed to compare the body weight at different times (* $p < 0.05$).

Results and discussion

Preliminary Phytochemical Screening

The ethanolic extract of *C. perglabra* obtained by the methods described in the methodology, produced a yield of 18% on a dry basis. Specific reactions to identify the presence of secondary metabolites (Shinoda test, Zn/HCl assay, Liebermann-Burchard reaction, Hager-Salkowski reaction and foam assay) were positive, showing that *C. perglabra* extract contains flavonoids, steroids, terpenoids and saponins (Table 2).

Chromolaena perglabra (B.L. Robinson) King & H. Rob, despite having a wide geographical distribution in Colombia, there are few studies that validate the traditional knowledge about their biological properties. There are some references that show

studies about its taxonomy, its biological activity and its phytochemical characterization, the latter are consistent with the findings of our research, specifically in the high content of flavonoids [26, 27].

Acute toxicity

The administration of the assayed doses of the complete ethanolic extract of *C. perglabra* intraperitoneally to ICR-CD1 mice (highest dose: 2000 mg / kg) did not cause the death of any animal. According to the Globally Harmonized System [25], *C. perglabra* extract was classified in the hazard category 5. The daily evaluation of the general state of the animals did not show behaviors or irregular states such as piloerection, ataxia, depression, changes in their eating habits, among others.

The weight of the animals was monitored daily for 14 days; no significant differences were found when comparing the weights of each experimental group and the control group (Figure 2; ANOVA, $p > 0.05$).

In the macroscopic evaluation of the extracted organs, splenomegaly was observed in the samples of all the treated groups with respect to the control group, with the exception of one of the mice of G1a that did not show alterations in the size of the organ; the rest of the organs showed a normal appearance (Table 3).

Histopathological study

I) Spleen: evidenced lymphocyte proliferation and loss of tissue architecture in the sections made for the different dilutions of *C. perglabra* extract administered with a final diagnosis of mild lymphoid hyperplasia.

II) Liver: showed a marked increase of inflammatory cells of acute (neutrophils) and chronic (lymphocytes) states of predominant location in the stroma of the organ in all the groups studied. The diagnosis was toxic hepatitis with areas of repair.

III) Stomach: the microscopic observation of the gastric gland showed no alterations in any of the groups.

IV) Kidney: the microscopic observation of the kidney showed no alterations in any of the groups.

The metabolites identified in our study have no history of toxicity, but they give the plant different properties that would explain their biological activity, among which flavonoids stand out for their antioxidant and anti-inflammatory activities, the

latter evaluated by different authors [28, 29]; this has allowed its use in the treatment of pathologies with complex etiologies, such as different types of cancer (breast, ovary, mouth, esophagus, colorectal, among others) [30-34], metabolic diseases such as osteoporosis and diabetes mellitus type 2, diseases of the central nervous system such as multiple sclerosis, dermatological, cardiovascular diseases, among others [35-39].

According to Zn / HCl assay, the presence of steroids was established, with recognized anti-inflammatory activity and suppressive of the immune system that has allowed its use in different medical specialties [40-42]. The terpenes obtained correspond to the monoterpene type; several articles have been published in which the cytotoxic, anti-inflammatory and antimicrobial activities were evaluated, [43-45]. Additionally, the identification of saponins agrees with the studies that report applications in the cosmetic and pharmacological field [46].

Although the scientific manuscripts published on *C. perglabra* are few, the results of the phytochemical analysis described in this study are comparable with the results published for other species such as *Chromolaena odorata* and *Chromolaena leptocephala*, species rich in phenolic compounds [47]. In Latin America, Colombia is a pioneer in the advancement of research on *C. perglabra*, with a good approximation to its chemical composition and biological activity [27].

With respect to acute toxicity findings, comparable information is not available in the literature; particularly on the genus, there are recent studies evaluating acute toxicity in *Chromolaena odorata*, however, only one of them showed toxicity at very high doses of obtained ethanolic and aqueous extracts (LD₅₀ > 5000 mg/kg and LD₅₀ > 2154 mg/kg, respectively), however, it was concluded as non-toxic [24, 48]. In this study, histopathological changes were identified in two of the four organs analyzed (spleen and liver) in all doses administered, but there was no evidence of lethality in the 72 hours after inoculation of the extract via IP. The experts considered the findings in the spleen mild. The macroscopic and microscopic morphology of the liver and stomach was normal.

In future research, it is recommended to perform subchronic toxicity studies (28 days) to evaluate the

safety of *C. perglabra* extracts administered at repeated doses, for longer periods.

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Table 1. Acute toxicity test.

	Weight (g)	Sex	Substance	Dose (mg/kg)	Route of administration	Volume (ml)
Group 1 (n=15)						
G1a	24,5	F	<i>C. perglabra</i> extract	125	Intraperitoneal	0,5
G1b	24,5	F	<i>C. perglabra</i> extract	250	Intraperitoneal	0,5
G1c	24,5	F	<i>C. perglabra</i> extract	500	Intraperitoneal	0,5
Group 2 (n=10)						
G2a	28,7	F	<i>C. perglabra</i> extract	1000	Intraperitoneal	0,5
G2b	28,7	F	<i>C. perglabra</i> extract	2000	Intraperitoneal	0,5
Group 3 (n=5)						
	24,5	F	Distilled water	NA	Intraperitoneal	0,5

F: female.

C. perglabra extract: *Chromolaena perglabra* extract.

NA: Not Apply.

ml: milliliter.

Table 2. *C. perglabra* preliminary phytochemical Screening.

Test	Result	Compound
Shinoda	+	Flavonoids
Zn/HCl	+	Flavonoids
Lieberman-Burchard	+	Steroids
Hager-Salkowski	+	Terpenoids
Foam index	+	Saponins

(>): Reactive.

Table 3. Macroscopic morphology.

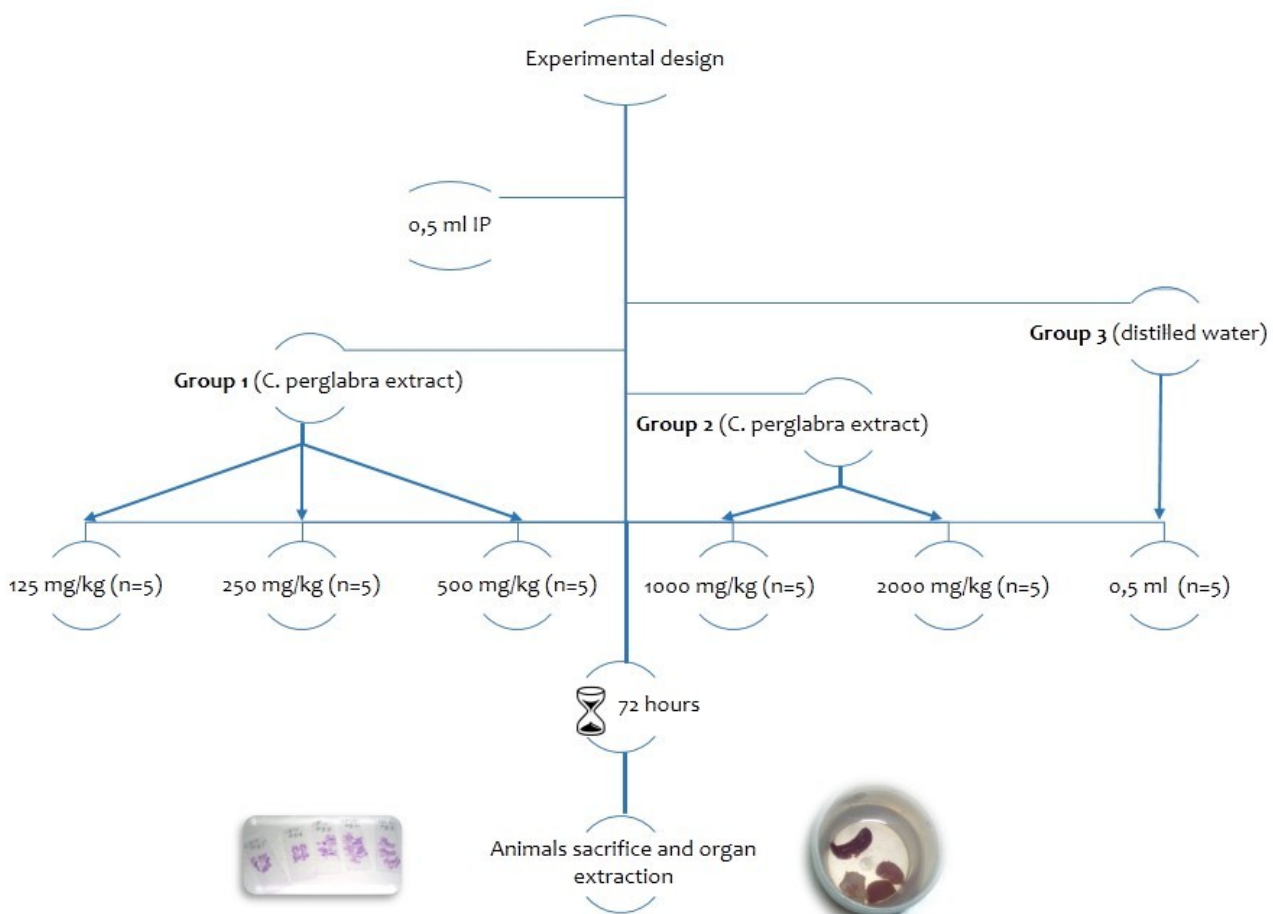
Animals	Dose (mg/kg)	Spleen	Liver	Stomach	Kidney
Group 1 (n=15)					
G1a*	125	↑	n	n	n
G1b	250	↑	n	n	n
G1c	500	↑	n	n	n
Group 2 (n=10)					
G2a	1000	↑	n	n	n
G2b	2000	↑	n	n	n
Group 3 (n=5)					
	NA	n	n	n	n

*: only one mouse of this group did not present modifications in its usual aspect.

↑: splenomegaly.

NA: Not Apply.

n: normal.



Group 2: *C. perglabra* extract IP at different doses. Group 3: control. (g): grams. (d): days.

Figure 1. Experimental design.

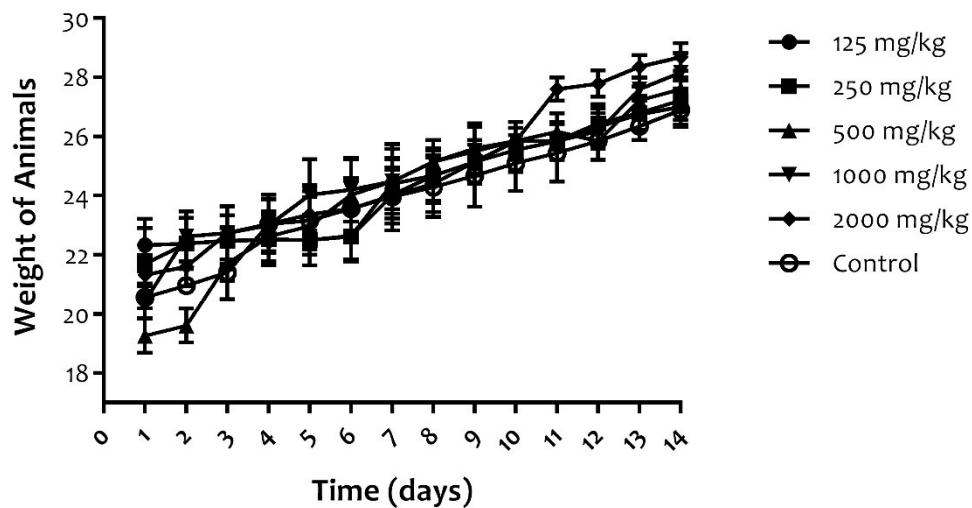
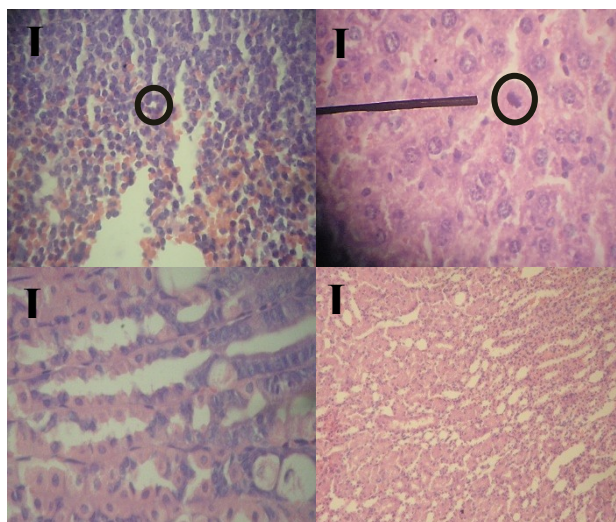


Figure 2. Behavior of the weight of the animals during 14 days after the administration of the *C. perglabra* extract.



Group 3 (control) did not present any alteration of the organs that were affected in groups 1 and 2 (not shown).

Figure 3. Histopathological study.