Animal Health

Original

Evaluation of Toxicity of a Single Oral Dose of *Dichrostachys cinerea* Extracts in Rats

Aida Yanet Borges Espinosa *, Osmany Marrero Chang **, Mirieisy Seijo Wals **, Aliette Martínez Díaz **, Leonel Lazo Pérez ***, Mario Reinoso Pérez ***

*Department of Animal Health, Villa Clara. Cuba.
**Toxicology Laboratory. Center of Chemical Bioactives The Marta Abreu Central University of Las Villas. Cuba.
***Department of Veterinary Medicine and Zootechny. Faculty of Agricultural Sciences. The Marta Abreu Central University of Las Villas. Cuba.

Correspondence: aybe5@nauta.cu

Received: June 2022; Accepted: July 2022; Published: September 2022.

ABSTRACT

Background: The utilization of edible biomass of *Dichrostachys cinerea* could become an effective alternative for the control of animal parasitosis. **Aim.** To evaluate the limit dose toxicity of an extract of legumes, reshoots, and leaves of adult *D. cinerea* plants in rats. **Methods:** The study comprised young-adult albino consanguineous and heteroxenic rats (*Rattus norvegicus*) of the Sprague Dawley (Cenp: SD) line, of both sexes, weighing between 150 and 200 g. Three aqueous extracts by 20% crushing from plant biomass, reshoots from the adult plants, and legumes were evaluated through an oral toxicity test with a limit dose of 2000 mg/kg body mass. Mortality and symptoms or signs of toxicity were controlled. A necropsy and macroscopic analysis of the animals at the end of the study was performed to detect lesions. STATISTICA, version 10, was used, and the Wilcoxon and Man Whitney U non-parametric paired tests were performed to compare the initial and final weights by sexes, and weight gains by sexes, respectively. **Results:** The rats from both sexes increased their body weight, demonstrating that the extract caused no negative effect. There were significant differences in weight gains between the males and females; no macroscopic lesions or pathological alterations associated with the administration of the product were observed. **Conclusions:** The aqueous extract of legumes, reshoots, and leaves of adult *D. cinerea* plants administered to rats orally was not toxic at the limit oral dose in rats.

Keywords: limit dose, legume, plant, reshoots (*Source*: *BVS*)

Citation (APA)
Borges Espinosa, A., Marrero Chang, O., Seijo Wals, M., Martínez Díaz, A., Lazo Pérez, L., & Reinoso Pérez, M. (2022). Evaluation of the Toxicity of a Single Oral Dose of Dichrostachys cinerea Extracts in Rats *Journal of Animal Production*, *34*(3). https://revistas.reduc.edu.cu/index.php/rpa/article/view/e4257

INTRODUCTION

In the last four decades highly effective antiparasitic synthetic molecules have been developed with a broad spectrum and low residual effects (Dorta-Contreras, 2007). However, today, the treatment of parasitic diseases has become difficult due to commercial drug resistance, which demands new alternatives to control these resistant or multi-resistant pathogens. One of these choices is the inclusion of secondary metabolites from plants with gastrointestinal antibacterial and/or nematode activity (Satyajit and Lutfun, 2012; Epe and Kaminsky, 2013; Medina *et al*., 2014; Hernández-Alvarado *et al*., 2018).

Several research studies have dealt with the pharmaceutical potential of several plants, such as *D. cinerea*, whose utilization is not well spread in Cuba. It is particularly effective to control gastrointestinal nematodes (Arece *et al*., 2012), so the utilization of edible biomass from this plant could become an effective alternative for the control of animal parasitosis in small ruminants.

Experimental toxicological and pharmacological studies constitute a step in the critical route that new products, nutraceuticals, etc. must take, so it can be scientifically established according to safety, consumption, and availability criteria (Repetto and Repetto, 2012). The utilization of biopreparations from D. cinerea require a characterization of toxicity.

Accordingly, the aim of this paper was to evaluate toxicity at a limit dose of an extract of legumes, reshoots, and leaves of adult *D. cinerea* plants in rats.

MATERIALS AND METHODS

The study was conducted at the Toxicology Laboratory from the Biological Department of Chemical Bioactives from The Marta Abreu Central University of Las Villas.

Identification of the assay substances and the excipient

Product name:

Aqueous extract of *D. cinerea* (L.) legumes

Aqueous extract of *D. cinerea* (L.) reshoots

Aqueous extract of adult *D. cinerea* (L.) leaves

Extract obtainment: Aqueous extract by crushing 20% plant biomass consisting of legumes, leaves from the adult plant, and the reshoots, collected in the same area and time of the year. The plant material was collected from the legumes, leaves, and reshoots of *D. cinerea*, established at EL Vaquerito Cooperative of Credits and Services, on Camajuaní Road km 2.5, Santa Clara, province Villa Clara, Cuba.

Organoleptic characteristics:

- Color: Dark brown-greenish
- Look: Fluid liquid.
- pH: 6.6

Assay system

Young *Rattus norvegicus* Berkenhout 1769 (albino) consanguineous and heteroxenic rats (150 g-200) of the Sprague Dawley (Cenp: SD) line, were used in the study. A digital technical balance (Sartorius) from the facility was used to check the weight of the animals. The females were nulliparous and non-gestating. The animal weight variations did not exceed ± 20% the weight mean for every sex.

The animals were assigned to the experimental group at random. At the end of the study, the animals were sacrificed by concussion. All the bioethical principles and procedures were taken into account to prevent unnecessary suffering.

The conditions of maintenance and feeding, lodging, and adaptation, along with the identification, cleansing, and disinfection procedures followed the rules for animal welfare. The drinking water was previously sterilized by autoclaving. Water and feed availability was supplied *ad libitum* throughout the study.

The following environmental conditions were met: Temperature: 22ºC ± 3ºC, relative humidity: 50-70%, photoperiods: 12 / 12 hours, and light intensity: 200 lux-250 lux. The climatic variants were kept through air conditioning, properly programmed automatic fluorescent light off/on system.

Distribution and pooling of the experimental groups

The animals (ten rats) eligible for the experiment were distributed to each experimental group at random. Each experimental group consisted of five rats of both sexes. The same-sex animals were lodged in Type T-3 polycarbonate cages with bagasse bedding throughout the experiment. The cages were labeled on the corresponding metal grid.

The experimental group design and formation is shown in table 1.

Table 1. Experimental groups

Groups	No. and sex	Dose
Leaf extract of the adult plant	5 females	2000 mg/kg body mass
	5 males	
Extract of reshoots	5 females	
	5 males	
Extract of legumes	5 females	
	5 males	

Administration route

The administration was oral, using a rigid steel straw, thicker on the end to prevent the perforation of the esophagus. The animals fasted the night before the experiment. In the morning, the animals were weighed and were given the experimental substance depending on their body weight. The animals fasted for three to four hours following the test, then they were fed again. The water was never withdrawn from the cages.

Dosage: The extract was administered at an internationally established dose for experimental products whose composition or previous experimental data will cause no o very little toxicity. The extract volume consisted of 2000 mg/kg body mass.

Clinical observations

The animals were observed for the first 3-4 hours following the administration; the purpose was to detect the occurrence of mortality or the emergence of symptoms or signs of toxicity. Then the observations took place one-two times a day, for 14 days.

Then, the animals were sacrificed via concussion to perform a microscopic pathological analysis.

Body weight

The animals were weighed at the beginning of the study and every 7 days upon administration, using a digital technical balance (Sartorius). The weight variations were calculated and recorded in cases of survival for over one day. Body weight gain was also evaluated until the post-administration observation period ended.

Anatomopathological study

A necropsy and macroscopic analysis of the animals was performed to detect lesions and macroscopic alterations at the end of the study.

Ethical considerations

The study relied on the principles of reducing the number of animals excluding the individuals in the control group, with the least number of rats included in this type of study. The staff in charge of the study had expertise in the use of laboratory mammals, and their work included the stress-reducing procedures, and the mitigation of distress, as well and the sanitary-hygienic state of the animals. Animal welfare was ensured by observing the conditions for the species, non-variability of responses, and the reliability of data gathered.

Statistical analysis

A Mann Whitney U non-parametric test was conducted to compare weight increase by sex at the beginning and end of the experiment. A Wilcoxon paired test was done to compare the initial and final weights by sex. The statistical significance was $p \leq 0.05$. The studies were done using STATISTICA, version 10.

RESULTS AND DISCUSSION

The trial included 15 clinically healthy rats of both sexes ready for the study, which did not have any mortality or clinical symptoms associated with the administration of the aqueous extract from the legumes, reshoots, and leaves from adult *D. cinerea* plants to the animals of both sexes.

The weight of the animals during the study increased in a logical way, according to the duration of the experiment.

Table 2 shows initial and final average weights, as well as the live weight gains, higher in the male animals that received the aqueous extract from legumes, reshoots, and the leaves of adult plants orally.

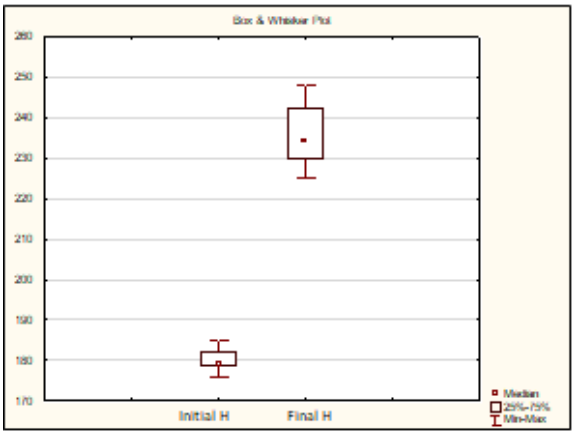
Table 2. Behavior of the average weight (SD±) in the rats during the oral toxicity study of the aqueous extracts of legumes, reshoots, and leaves from adult *D. cinerea* plants

Treatments	n	Sex	Initial weight (g)	Initial weight (g)	Weight increase (g)
Legumes	5	Males	199.6 ± 13.97	235.8 ± 9.23	135 ± 8.15
	5	Females	180.2 ± 3.42	234.6 ± 17.31	55.6 ± 8.50
Reshoots	5	Males	199.8 ± 5.89	329.6 ± 12.34	129.8 ± 7.82
	5	Females	176.4 ± 8.82	239.6 ± 9.50	63.2 ± 10.25
Leaves from the adult plant	5	Males	204.8 ± 6.94	311.2 ± 11.45	106.4 ± 11.08
	5	Females	202.4 ± 8.56	250.6 ± 12.81	48.2 ± 6.30

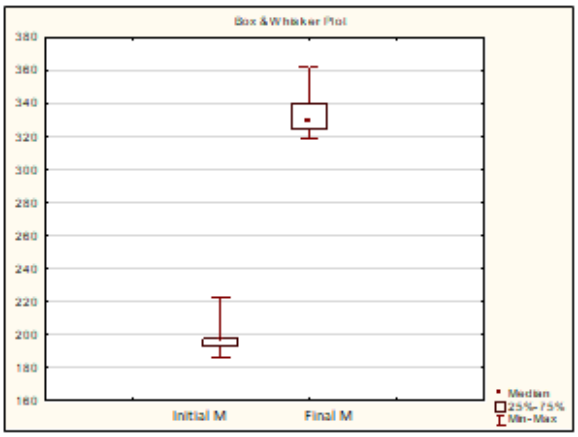
The statistical analysis of body mass development in the rats that received the extract evidenced the existence of significant statistical differences ($p \leq 0.05$), in relation to the initial and final weights by sex. In the two sexes, weight was significantly higher at the end of the study, demonstrating a positive increase of this health indicator during the three evaluations (Graphic 1).

Extract of legumes

a) Behavior of females (Wilcoxon Matched Pairs Test, $p = 0.043$)

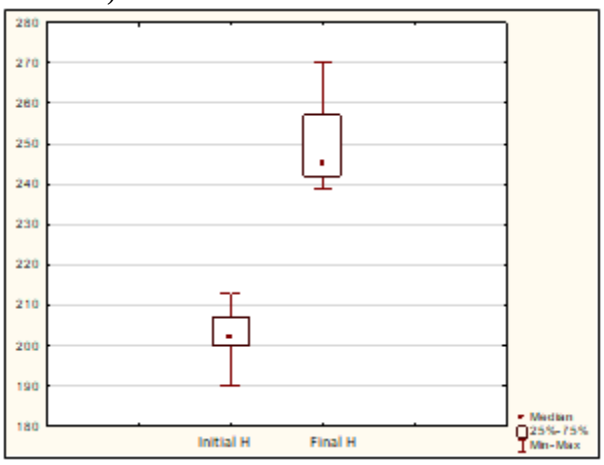


b) Behavior of males (Wilcoxon Matched Pairs Test, $p = 0.043$)

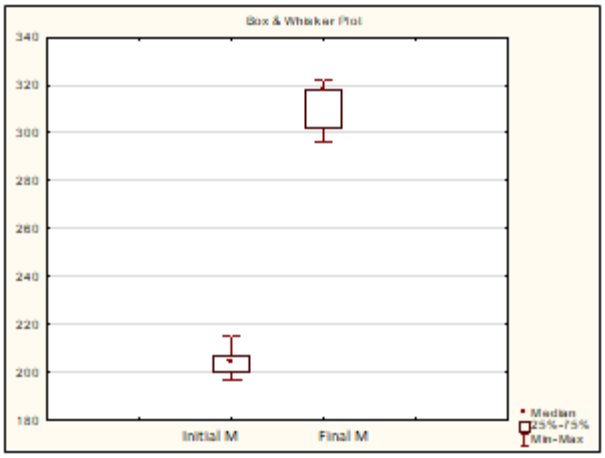


Adult plant leaf extract

c) Behavior of females (Wilcoxon Matched Pairs Test, $p = 0.043$)

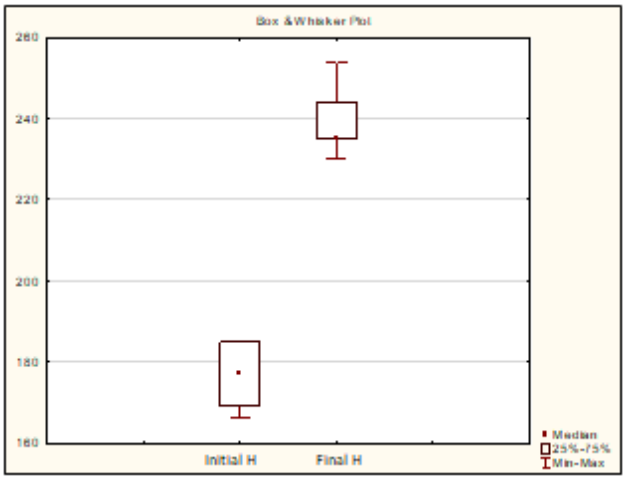


d) Behavior of males (Wilcoxon Matched Pairs Test, $p = 0.043$)

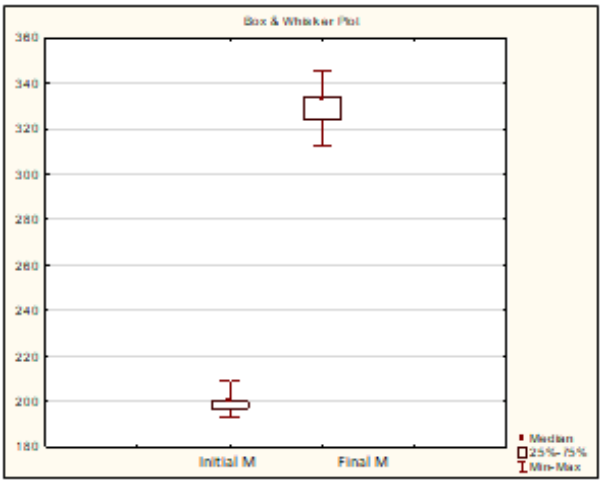


Reshoot extract

e) Behavior of females (Wilcoxon Matched Pairs Test, $p = 0.043$)



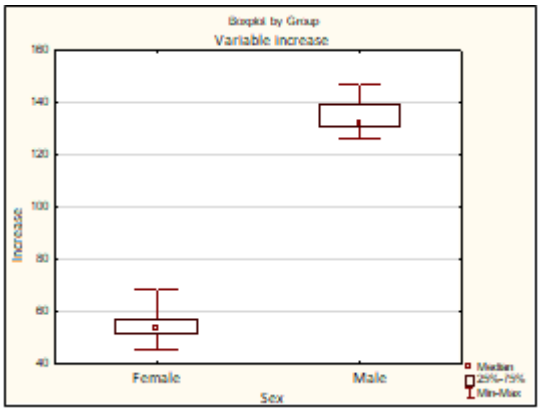
f) Behavior of males (Wilcoxon Matched Pairs Test, $p = 0.043$)



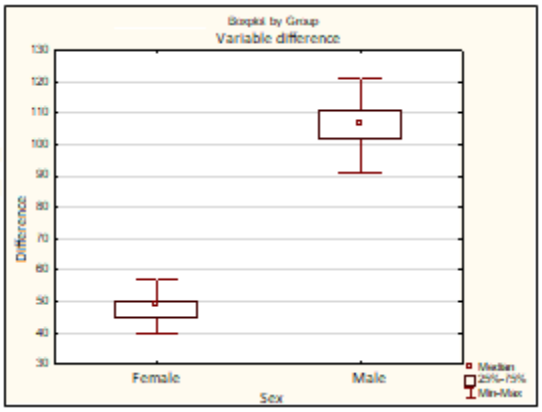
Graphic 1. Analysis of body weight behavior in the rats that received *D. cinerea* extracts. Comparison of the weight by sex at the beginning and end of the study.

The comparative analysis of the effects of the three extracts on body weight increase by sex demonstrated the existence of significant differences ($p \leq 0.05$), that favored the male rats (Graphic 2).

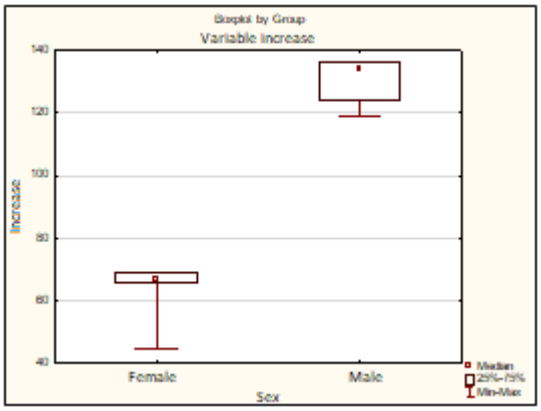
a) Extract of legumes



b) Adult plant leaf extract



c) Extract of reshoots



Graphic 2. Analysis of body weight increase in the rats that received *D. cinerea* extracts. Sex comparison (Wilcoxon Matched Pairs Test, $p = 0.012$).

Overall, all the rats increased their body weight significantly at the end of the study, a normal behavior for the somatic development period, which demonstrates the positive influence of extract administration.

There were significant differences in terms of weight increases between the males and females, a normal behavior for this species and line of rats. This weight behavior was in correspondence with the age of the individuals and sex differences, which was corroborated when comparing the weights reported by the Animal Resource Center, Department of Rodent Reference Information, for rats of the same line and age (Animals Resources Centre, 2021).

The necropsies performed to all the animals sacrificed at the end of the exposure period did not show macroscopic lesions with pathological alterations, or attributed to the administration of the product.

Several researchers referred to the therapeutical effects of *D. cinerea* used as alternative medication (healing, emetic, anti-inflammatory, and vermifuge), through different forms and parts of the plant (Borges *et al*., 2020).

The broad use of different ways of preparation with different parts of the *D. cinerea* plant complements the absence of toxicity that can be demonstrated with the administration of limit doses of the aqueous extract, so it is safe for the expected use.

CONCLUSIONS

The aqueous extract of legumes, reshoots, and leaves of adult *D. cinerea* plants administered to rats orally was not toxic to the limit oral dose in rats.

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AUTHOR CONTRIBUTION STATEMENT

Research conception and design: AYBE, OMC, MSW, AMD, LLP, MRP; analysis and interpretation of data: AYBE, OMC, MSW, AMD, LLP, MRP; redaction of the manuscript: AYBE, OMC, MSW, AMD, LLP, MRP.

CONFLICT OF INTEREST STATEMENT

The authors declare the are no conflicts of interest.