# TOXICOLOGICAL PRE-CLINICAL EVALUATION OF THE FLUID EXTRACT OF ISOCARPHA CUBANA BLAKE

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## Summary

The common chamomile (Isocarpha cubana Blake) is a plant commonly used by our people. A few studies have been carried out about it and none has demonstrated that the plant is sufficiently harmless. Therefore, a pre-clinical toxicological study on the fluid extract of the plant is proposed as an indispensable preamble for experimentation in human beings. In this regard the trials on oral and dermal acute toxicity were developed. All the trials on irritability were also carried out. They included dermal, ophthalmic, rectal, vaginal and oral mucus trials and were aimed at evaluating the irritant potential of the plant extract while in contact with the different corporeal structures in one way or another. All the trials were carried out by following the protocols internationally established (ISO, OECD). The fluid extract provoked oral acute toxicity, with death in animals at limit dose and clinical signs of toxicity at a lesser dose. From a dermal point of view, no sign of toxicity was presented and there was weight gain in animals. It provoked slight macroscopic alterations in the oral mucus, no dermal irritation at all, severe damage in the ocular structures and macroscopic alterations in the rectal and vaginal mucus. No test of irritability presented any other clinical sign. The fluid extract presented oral acute toxicity and was harmless from a dermal point of view. It is a non-irritant product for the oral mucus, slightly irritant by the rectal and vaginal ducts and severely irritant for the ocular structures.

**Key words**: toxicology, Isocarpha, preclinical, irritability.

## Introduction

The common chamomile (Isocarpha cubana Blake) is a wild plant of our fields, which is said to have the same properties of the sweet chamomile (Matricaria recutita, L.). The anti-inflammatory and anti-diarrheic effect of its fluid extract has been demonstrated pre-clinically. There were no previous studies which showed that it was not toxic, therefore, a pre-clinic toxicological study on this product was proposed as indispensable for its further evaluation in human beings, production, commercialization and use in our health system.

The trials about oral and dermal acute toxicity leads to the evaluation of the innocuousness of the preparation, since they are the two ways proposed for its administration. The evaluation of the irritability is carried out with the objective of determining the irritating potential of the extract when it gets in contact with the different body structures.

## Materials and methods

The toxicological evaluation of the fluid extract of the plant was carried out by means of pre-clinical tests based on norms established internationally (OECD, ISO), evaluating the oral and dermal acute toxicity, the dermal and ophthalmic irritability, and oral, vaginal and rectal mucus irritability. The fluid extracts of the common chamomile were prepared from the well identified, dried and treated plant, using a hydro-alcoholic solution at 70% as solvent. They were tested chemical and microbiologically, according to the nationally established norms and guaranteeing the necessary requirement for its evaluation.

### Evaluation of the oral acute toxicity

First, a pre-clinic trial at a limited dosage was carried out, using the procedures described in the OECD (Organization for Economical Cooperation and Development) protocol [(1)]. The test was developed at 2000 mg/kg, using Wistar rats, with a minimum of 5 animals for trial for sex. The product was received by males and females, which formed 2 groups for the purpose. Taking into account the results obtained from this test, it was decided to carry out the trial by means of the methods of the classes. With this method, the dosage lowers and classification of the European Community for the chemical substances is used, according to the OECD norms [(2)]. In this case, the trial is at 1000 and 500 mg/kg. In the observation, signs of retarded toxicity were included. The rats were weighed after 1, 7 and 14 days. Their organs were observed to find out affectations, and samples were taken for there histopathological process. The rats weigh at the different periods of time were processed statistically by means of the analysis of variance of one way of classification and its posterior application of Student Newman Keuls test ($p<0.05$).

**Evaluation of dermal acute toxicity**
The procedures were according to the international norms of OECD[3], OPPTS 885.3100[4], EPA I TIER[5]. Wistar rats were used in the test. Two groups of 5 rats each were formed, divided according to sex, using the product in both of them. The trial was carried out at a limited dosage of 2000 mg/kg. Frequent observations were done during the first day, and one a day until the 14 days of the experiment. The clinical signs of the main macroscopical system were registered systematically, also the behaviour of the animals weigh was evaluated and processed statistically by means of an analysis of variance of one way of classification and after that, with the Student Newman Keuls test, with a $p<0.05$.

**Evaluation of the oral mucus irritability**
It was carried out according the ISO norm [6], using golden Sirius Hamster, distributed, at random, in 3 groups: one for control (without treatment), a group treated with placebo, and a third group treated with the extract being studied. The product was placed for a period of 24 hours of time, daily treated in 14 days. The exposed sites were evaluated using the Draize table [7], the ISO table for the oral mucus [6] and the calculation of the irritability index was led following the requirements of the ISO [6].

**Evaluation of the dermal irritability**
The trial described by the OECD and Draize was used, among others [7,8,9,10]. The trial was developed in rabbit, using males from $F_1$ line. The animals were examined to find signs of erythema and edema after an hour, 24, 48 and 72 hours of the plaster removal. The dermal irritation was registered according to the table described by Draize in the norm of the OECD [9]. The calculation of the index of primary irritation (IPI) was carried out taking into account the results obtained, according to the ISO [8].

**Evaluation of the ophthalmic irritability**
In this case, the Test of Ocular Irritability [6,10], established internationally, was applied to males rabbit of $F_1$ line. Judgement of the ocular structures (cornea, conjunctiva and iris) were done at 1, 24, 48 and 72 hours after the application of the product being studied, making the readings following the Draize scale [12] and after that, the index of ocular irritation was calculated by the scale of classification of García and col. [13].

**Evaluation of the rectal irritability**
This test was carried out following the ISO 1093 norm [8], in male albino rabbits. Three groups of 4 animals each were formed: a control group (without treatment), a group treated with placebo, and a third group treated with fluid extract of common chamomile. The product was administered every 24 hours during 14 days. 24 hours after the initial application and immediately before each treatment it was carried out an evaluation of the perineum appearance for discharge signs, erythema and irritation [7].

The evaluation and the calculation of the irritability index were carried out following the ISO indicators.

**Evaluation of the vaginal irritability**

This test was carried out following the ISO 10993 norm [8], in female albino rabbits. Two groups of 4 animals each were formed: a control group (without treatment), and a group treated with fluid extract of common chamomile. The procedure was similar to that of the rectal irritability test, but evaluating, in this case, the vaginas. In all the toxicity and irritability tests it was followed the histopathological processing when macroscopic sings of alteration were found.

## Results

In the evaluation of the acute oral toxicity, at the dose of 2000 mg/kg, the fluid extract of common chamomile produced observable toxicity and death in laboratory animals. The samples taken from the selected organs did not present affects from a macroscopic point of view what lead to not develop the histopathological processing. At the dose of 1000 mg/kg, the first day they presented clinical signs in the respiratory system as well as sedation and drowsiness. No animal died. At 500 mg/kg no clinical signs were reported in either of the two studied groups. In the evaluation of the acute dermal toxicity no alteration of the evaluated aspects was observed in either of the observations. The behaviour of body weight in both trials is showed at next.

**Table # 1: Behaviour of body weight (g) in the acute oral toxicity test.**

| Group | Time (days) | | | | | |
|---|---|---|---|---|---|---|
| | At 1000 mg/kg | | | At 500 mg/kg | | |
| | 1 | 7 | 14 | 1 | 7 | 14 |
| ***Males*** | 255.3 ± 23.6 a | 285.0 ± 22.9 a | 358.4 ± 20.2 b | 206.6 ± 2.08 a | 221.6 ± 6.66 b | 232.0 ± 10.1 b |
| ***Females*** | 205.6 ± 20.4 c | 225.2 ± 16.3 cd | 237.0 ± 10.5 d | 217.6 ± 2.52 c | 231.6 ± 9.02 cd | 244.6 ± 9.45 d |

Different letters indicate significant differences ($p<0.05$)

**Table # 2: Behaviour of the body weight (g) in the acute dermal toxicity test**

| Group | Time (days) | | |
|---|---|---|---|
| | 1 | 7 | 14 |
| ***Males*** | 241.0 ± 11.96 a | 271.0 ± 9.19 b | 320.2 ± 14.26 c |
| ***Females*** | 202.6 ± 6.88 d | 221.08 ± 8.93 de | 234.6 ± 14.59 e |

Different letters indicate significant differences ($p<0.05$)

The results of the evaluation of the oral mucus irritability are showed in the Table # 3.

**Table # 3: Behaviour of the irritability evaluation in the oral mucus**

| Group | Epithelium | Congestion | Edema | Leucocytes |
|---|---|---|---|---|
| *Control* | 0 | 0.66 | 0 | 0.0 |
| *Placebo* | 0 | 1.0 | 0 | 0.33 |
| *Treated* | 0.66 | 1.0 | 0 | 0.33 |

In the evaluation of the dermal irritability no reaction of erythematous or edematous kind was observed. Other important clinical signs were not presented. The results of the evaluation of the ophthalmic irritability are showed in the Table # 4.

**Table # 4: Behaviour of the evaluated aspects for the ophthalmic irritability**

| Day | Conjunctiva | | | Cornea | | Iris | | Total |
|---|---|---|---|---|---|---|---|---|
| | Erythema | Edema | Secretion | Area | Opacity | + | - | |
| 1 | 3 | 4 | 3 | 4 | 4 | 10 | - | 110 |
| 2 | 3 | 3 | 3 | 4 | 4 | 10 | - | 110 |
| 3 | 3 | 2 | 3 | 3 | 4 | 10 | - | 86 |
| 4 | 3 | 2 | 3 | 3 | 4 | 10 | - | 86 |
| TOTAL | | | | | | | | 392 |

The results of the evaluation of the rectal and vaginal irritability are showed in the Table # 5 and 6.

**Table # 5: Behaviour of the evaluated aspects for the rectal irritability**

| Group | Epithelium | Congestion | Edema | Leucocytes |
|---|---|---|---|---|
| *Control* | 0/0/0* | 0/0/0 | 0/0/0 | 0/0/0 |
| *Placebo* | 0/0/0 | 0/0.75/0.5 | 0/0/1 | 0/0/0.25 |
| *Treated* | 0.5/0.75/1.25 | 0.5/0.75/1.25 | 0.5/0.5/1.25 | 0.25/0.25/0.75 |

**Table # 6: Behaviour of the evaluated aspects for the vaginal irritability**

| Group | Epithelium | Congestion | Edema | Leucocytes |
|---|---|---|---|---|
| *Control* | 0.25/0.5/0* | 0.5/0.75/1 | 0/0/0 | 0/0/0 |
| *Treated* | 0.25/1.25/1.25 | 0.75/1.75/2.25 | 1.5/1/1.75 | 1/1.25/1.5 |

* Irritability Evaluation of Proximal portion / medial portion / distal portion

The irritation index obtained in the five trials of irritability is: 0.33 for the oral mucus (0.11 the placebo); 0 for the skin; 98 for the ophthalmic structures; 3.11 for the rectal mucus (0.83 the placebo) and 4.11 for the vaginal mucus.

## Discussion

The fluid extract resulted toxic by oral way at a limit dose and this toxicity decreased in proportion as the dose diminished. At 1000 and 500 mg|kg there was a gain in weight in the animals (Table # 1); this wasn't statistically significant in some cases. This proved the decrease in toxicity. According to the classification by the European Union, the fluid extract of the Common Chamomile is considered harmful. We consider that the results of the acute oral toxicity can be influenced by the hydro-alcoholic composition of the fluid extract. This means that the presence of ethanol can be providing symptoms of toxicity independent from those of the plant. From a dermal point of view, the fluid extract resulted harmless and some weight gain is obtained in test animals (Table # 2). It is so considered non-toxic for human beings from an acute point of view.

When evaluating the irritability in the oral mucus certain light alterations from a macroscopic point of view were presented (Table # 3). In the groups treated the evaluation obtained for the irritation index was between 0 and 1, so it is considered non-irritant for the studied structure, according to the ISO norm. The product passed satisfactorily the test of dermal irritability and is classified as non-irritant. The ocular damages were severe for the three studied ophthalmic structures (Table # 4). The index of ocular irritation was 98. According to this result it is classified as severe irritant when using the García and Col classification chart. When evaluating the rectal and vaginal irritability alterations in these mucus membranes from a macroscopic point of view were presented (Table # 5 and 6). This implied its histopathological processing. In the case of the rectal irritability, the evaluation obtained from the irritation index for the group treated was between 1 and 4 and so it is considered as slightly irritant for the studied structure, according to the ISO norm and potentially non-irritant for man. For the vaginal mucus, according to the value obtained from the irritation index, the product is classified as moderately irritant, according to the ISO norm.

## Conclusions

The fluid extract presented oral acute toxicity and was harmless from a dermal point of view. It is a non-irritant product for the oral mucus, slightly irritant by the rectal and vaginal ducts and severely irritant for the ocular structures.

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