IJFDC
International Journal of Food, Drug and Cosmetics
January 2025 Vol 1 Issue 1 **https://doi.org/10.31674/ijfdc.2025.v1i01.001**

Evaluation for Anti-inflammatory activity of 100% Calabash Fruit (Crescentia cujete) extract on ICR Mice

Alao Dauda Isiaq

Faculty of Pharmacy ,Lincoln University College, Kuala Lumpur, Malaysia

Corresponding Author: Izyworld1@gmail.com

Abstract

This study evaluated the anti-inflammatory activity of 100% Calabash fruit (Crescentia cujete) extract on Institute for Cancer Research Mice. Crescentia Cujete is also known as Calabash and is a member of the Bignoniaceae family. Extract was obtained by macerating the fruit pulp without seeds in 95% ethyl alcohol for 72 hours with slight agitation. The evaporation process was done through the use of a rotary evaporator to obtain the pure extract. Calabash fruit extract was studied on egg-albumin induced paw edema (10mg/1000ml x BW) on ICR Mice. The Positive control, Diclofenac Sodium; and the extract was administered orally using a gavage to each of the ICR mice. Extract was administered to the Positive control group 30 minutes after inflammation. While the Negative control group was given water 30 minutes after inflammation. Paw measurements were taken every 30 minutes for 8 hours using the cotton thread method. Results obtained from the experimental control (mean 3.46cm) showed there was a significant decrease (mean 2.11cm) in inflammation on the egg-albumin paw edema on ICR Mice over the 8 hour observation Period. Unlike the negative control group (mean 2.78cm) which did not decrease as significantly (mean 2.26cm). In conclusion, the calabash fruit (Cresentia cujete) showed significant anti-inflammatory properties in reducing inflammation on egg-albumin induced paw edema on ICR Mice.

Key Terms: ANOVA – Analysis of Variance, Anti-Inflammatory, Calabash, Crescentia cujete, Egg-Albumin Induced Paw Edema, Inflammation.

Introduction

The study aimed to evaluate the potential anti-inflammatory activity of 100% ethanol extract of Calabash fruit on ICR mice. It aimed to assess the mean circumference of the ICR mice paw during the treatment of the following treatment groups:

1 Positive control Group (Egg albumin-induced paw edema on ICR mice treated with

Diclofenac Sodium)

2 Negative control group (Egg albumin-induced paw edema on ICR mice treated with

Distilled water)

3 Experimental group (Egg albumin-induced paw edema on ICR mice using 100% Calabash fruit extracts)

Also determine the significant differences among the treatment groups in terms of body score, appearance score, attitude score, and the presence of anti-inflammatory activities on ICR mice.

The Calabash species was obtained from Busay, Mountain View Nature's Park, Cebu City, Cebu, Philippines by Cecilia Maglasang, a General Service employee of University of Southern Philippines Foundation. This is due to her close proximity to the area where the Calabash is used as a decorative plant at the park. Authentication was done at the University of San Carlos – Biology Department, which is acknowledged by the Commission on Higher Education (CHED) a Canter of Development in both Marine Science and Biology.

Significance of the Study

This study aims to evaluate the ability of the Calabash fruit extract's properties to treat inflammation. This research will not only benefit the researchers, but the whole world in search for an herbal anti-inflammatory agent.

REVIEW OF RELATED LITERATURE

According to many published journals, Crescentia cujete is from the plant family of Bignoniaceae or the Jacaranda Family [1](Rahmatullah et.al., 2010). It is native to the tropical and subtropical regions of America. The place of origin is uncertain because it was cultivated in the Yucatan Peninsula since Pre-Hispanic times. This species grows naturally in the Caribbean islands and from Mexico through Central America to the northern regions of south America.[2](Yin et.al.,2012). Today, this plant is cultivated as a backyard tree in several countries across the world.

The objective of this study is to collect extracts, specifically ethanol extracts, from the fruit of Crescentia cujete Linn., and to identify if there are any presence of anti-inflammatory activities in the extract.

Materials and Methods

Male or female ICR mice with a body weight between 24-32 grams are used. The animals were starved for 12 hours. The mice were then injected with 0.1ml undiluted egg albumin into the sub plantar tissue of the hind paw and measurements are taken immediately via cotton thread method. 30 minutes later, the mice were given 100% Calabash extract orally via gavage. The circumference of the paw was then measured every 30 minutes in a duration of 8 hours[3] (Amabeoku, et.al., 2015; Yoon, & Lee, 2017).

Preparation of the extract was done at the Laboratory or Pharmacy Ground Floor of the Oscar Jereza Hall, University of Southern Philippines Foundation, Salinas Drive, Lahug, Cebu City. In this facility, the essential laboratory apparatuses were provided. The laboratory was clean, well ventilated and accessible.

The experiment was performed under certain laboratory conditions st the Pet Science Clinic, Kalubihan, Talamban Cebu City. The room where the research animals were held, was equipped with instruments needed for the study. Such instruments are, analytical balance, air- conditioner, beakers, syringes, and appropriate cages for the animals.

Results and Discussion

The evaluation of 100% Calabash fruit (Crescentiacujete) has proven that anti-inflammatory activity exists. The researchers rejected the null hypothesis and were able to conclude that 100% Calabash fruit (Crescentia Cujete) extract infeed has anti-inflammatory activity based on the results of the data observed throughout the study.

Study of particular photochemical constituents found in the calabash fruit (Crescentia cujete) that specifically possess anti inflammatory activate.

1. Assess the mean body weight, body score, attitude, and appearance of the ICR mice before the treatment:

Treatment Groups	Mean Body Weight	Mean Body Score	Mean Appearance	Mean Attitude
Experimental Group	28.378	4	4.86	4.66
Positive Control Group	32.61	3.84	4.77	5
Negative Control Group	28.56	4.04	5	4.69

The assessment of the treatment groups according to their mean body weight, score, appearance and attitude)

Results of the Mean Body Score of each group are as follows: Experimental group, 4; Positive Control group, 3.84; and the Negative Control group, 4.04. All mice are over-conditioned, meaning all mice were well-fleshed and the bones cannot be felt when pressure is applied.

The results of the Mean Appearance Score of each group are as follows: Experimental group, 4.86; Positive Control group, 4.77; Negative Control group, 5. All scores of each group interprets that all mice had skin tents present on the dorsum.

Results of the Mean Body Attitude are as follows: Experimental group, 4.66; Positive Control group, 5; and Negative Control group, 4.69. Only the Positive control group showed normal behavior. The mice were active in the cage prior and during handling. As for the Experimental and Negative Control groups, the mice had less activity but were alert and responsive during handling.

As a whole, the mice had an average body score of 3.96, average appearance score of 4.87, and an average score of 4.78. All of these indicate that the mice were in optimal conditions, normal body appearance, and active when handled by the researchers, although a decrease in mice activity was exhibited.

2. Determine the mean baseline circumference before the egg albumin-induced inflammation in the following groups:

Treatment Groups	Mean Circumference (cm) of paw Before the induction of Inflammation	Mean circumference (cm) of paw After the induction of Inflammation	Mean Inflammation (cm)
Experimental Group	1.64	3.46	1.82
Positive Control Group	1.71	3.37	1.66
Negative Control Group	1.8	2.78	0.98

The assessment of the Mean Baseline Circumference of the paws Before and After the egg albumin-induced inflammation)

From the data provided the researchers were able to prove that there was no inflammation prior to the injection of the phlogistic agent for any of the treatment groups. The Experimental group has an average circumference of 1.64cm before injection and had an average circumference of 3.46cm after injection. Mean inflammation obtained from the Experimental group was 1.82cm. the average circumference obtained from the Positive Control group was 1.71cm before injection and has an average circumference of 3.37cm after injection. 1.66cm was the average mean inflammation gathered form the Positive Control group. The average circumference from the Negative Control group was 1.8cm before injecting and had an average circumference of 2.78cm after injection. The Negative Control group had obtained a mean inflammation of 0.98cm. This data will serve as the baseline for the respective treatment groups.

3. Assess the mean circumference of the ICR mice paw during the treatment.

Treatment Groups	**Mean Circumference (cm) of paw After the induction of Inflammation**	**Mean circumference reduction (cm) of Inflammation**	**Mean Reduction (cm)**
Experimental Group	3.46	2.11	1.35
Positive Control Group	3.37	2.10	1.27
Negative Control Group	2.78	2.26	0.52

The assessment of the Mean Baseline Circumference of the paws during treatment)

The results show that they experimental group obtained an average of 2.11cm

throughout the treatment and observation period. The paw decreased within this period and had a mean reduction of 1.35cm. the Positive Control group had an average circumference of 2.1cm and a mean reduction of 1.27cm. Lastly, the Negative Control group had an average circumference of 2.26cm and a mean reduction of 0.52cm.

Determine the significant differences among the treatment groups in terms of body score, appearance score, attitude score, body weight and the presence of anti-inflammatory activities on ICR mice. The ANOVA otherwise known as Analysis of Variance shows the three therapeutic groups in reducing in inflammation by establishing a difference. To determine the difference Post-Hoc Analysis is needed.

ANOVA								
Source of Variation	SS	df	MS	F	P-value	F Crit	Decision	Interpretation
Between Groups	1.082	2	0.541	16.33	0.000709	4.103	Reject the Null	There is a significant Difference
Within Groups	0.331	10	0.033					
Total	1.414	12						

The ANOVA chart detailing the differences in reducing inflammation among the three therapeutic groups)

Post-Hoc Analysis: Using Tukey's Honestly Significant Difference

Treatment Groups Mean	Means Subtracted Between Two Groups	Difference Between Two Means	Tukey's Honestly Significant Difference (HSD)	Interpretation
Negative Control (2.71) Minus Positive Control (2.10)	2.71 – 2.10	0.61	0.377	**The Difference is Significant**
Negative Control (2.71) Minus Experimental (2.12)	2.71 – 2.12	0.59	0.377	**The Difference is Significant**
Positive Control (2.10) Minus Experimental (2.12)	2.10 – 2.12	0.02	0.377	**The Difference is NOT Significant**

Tukey's Honestly Significant Difference method applied to the study)

From the data gathered from the previous tables, the researchers needed to determine as to what degree each group differed from each other. By using the Tukey's Honestly Significant Difference method, the researchers concluded that the 100% Calabash fruit (Crescentia cujete) has no significant difference when compared to the Positive Control group.

According to the rule of Tukey's Honestly Significant Difference, if the mean difference is greater than the value of HSD, the difference is significant. From the Post-Hoc analysis data, it has been observed that when comparing the Negative Control group to the Positive Control group a difference of 0.61 was obtained. This value is greater than the HSD, indicating that the difference is significant.When comparing the Negative Control group to the Experimental group being compared with the Positive Control group in a way where the difference is significant. Unlike when the Positive Control group and Experimental group were compared, they had a difference of 0.2. This difference is less than the HSD which makes the difference not significant.

This means that the 100% Calabash Fruit extract results are comparable to the Positive Control group. By bring comparable, it simply mean that the Experimental group has similar results to the Positive Control group which took Diclofenac sodium. Unlike the Negative Control group, which has a significant difference between both Experimental and Positive Control group, the results are not comparable.

Reference

Rahmatullah, Mohammed, Walied Samarrai, Rownak Jahan, Shahnaz Rahman, Z U M Emdad Ullah Miajee, Majeedul H Chowdhury, Sazzadul Bari, A B M Anwarul Bashar, A K Azad, and Shamima Ahsan. "An Ethnomedicinal , Pharmacological and Phytochemical Review of Some Bignoniaceae." DNA Repair 4, no. 78 (2010): 236–53.

Szurpnicka, Anna, Anna Kowalczuk, and Arkadiusz Szterk. Biological Activity of Mistletoe: In Vitro and in Vivo Studies and Mechanisms of Action. Archives of Pharmacal Research. Vol. 43. Pharmaceutical Society of Korea, 2020. https://doi.org/10.1007/s12272-020-01247-w.

Latin American Agriculture: Prospects and Challenges, 2019. https://doi.org/10.1787/b2b742eb-en.

Akah, P. A., and Nwanbie, A. (1994). Evaluation of Nigerian traditional medicines plants used for rheumatic (inflammatory) disorder. J. Ethnopharmacol., 42: 179-182

Altman, R., Bosch, B., Brune, K., Patrignani, P., & Young, C. (2015). Advances in NSAID development: evolution of diclofenac products using pharmaceutical technology. Drugs,75(8), 859-877

Hess, S. M., and R. C. Milonig. "Assay for anti-inflammatory drugs." Inflammation, Mechanisms and Control. Academic Press, New York (1972): 1-2.

IJFDC
International Journal of Food, Drug and Cosmetics
January 2025 Vol 1 Issue 1 **https://doi.org/10.31674/ijfdc.2025.v1i01.001**