CHARTULAE JOURNAL
OFFICIAL DEPARTMENTAL RESEARCH PUBLICATION OF THE COLLEGE OF PHARMACY
ISSN:3082-4354 MAIDEN ISSUE VOL. 1 NO. 1

EVALUATION OF THE ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC LEAF EXTRACT OF KADPAAYAN (*PSYCHOTRIA LUZONENSIS*) IN EGG WHITE INDUCED INFLAMMATION IN ALBINO RATS

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ABSTRACT

Inflammation is a vital part of the body's immune response. Without inflammation, wounds would fester, and infections could become deadly. This study focused on the evaluating the anti-inflammatory activity of Kadpaayan (Psychotria luzoniensis) in different concentrations of the leaf extract: 100%, 75%, 50%, and 25%, with the used of egg white- induced rat paw edema. A plant sample was collected at Tawi, Penablanca, Cagayan, around 7:00-9:00am in the morning. Leaves were washed and air-dried for 7 days at room temperature. The dried leaves were macerated using 70% diluted alcohol for 72 hours. After filtering extracts and the evaporation of solvent, they undergo phytochemical screening, where the secondary metabolites present are coumarins, saponins, and phenols, which exhibit anti-inflammatory activity. The prepared concentration was compared to the positive control (Diclofenac), an anti-inflammatory drug available in the market. A total of thirty (30) albino rats of both sexes were selected in this study, with six (6) treatments having five (5) replicates each. To induce inflammation, 0.02 ml of egg white were injected into each of the rats' paws, and water volume displacement were used as a method. The concentration treatment and standard drug were administered orally after thirty (30) minutes of induction. Data showed that all concentrations have potential for anti-inflammatory activity, but 25% and 100% concentrations exhibited the greatest anti-inflammatory activity, as inferred from the progressively lower displacement values. The results showed that there is no significant difference in the anti-inflammatory activity of different concentrations of Kadpaayan (Psychotria luzoniensis) leaf extract and the positive control. Therefore, it concludes that Kadpaayan leaf extract is comparable in its ability to prevent inflammation against egg white-induced paw edema in albino rats.

Key words: *Psychotria luzoniensis, Egg white-induced paw edema, Diclofenac, Inflammation*

INTRODUCTION

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Inflammation, characterized by symptoms such as redness, swelling, warmth, and discomfort, is a crucial immune response to various irritants, including pathogens or physical injuries.

In addition, chronic inflammation contributes significantly to morbidity and mortality worldwide, highlighting the need for effective and safer anti-inflammatory treatments. Traditional anti-inflammatory drugs like NSAIDs are widely used but often cause adverse effects such as gastric ulcers and organ toxicity, prompting exploration into alternative therapies such as medicinal plants. Despite the prevalence of available anti-inflammatory medications, significant gaps remain in the quest for treatments that are both effective and safe. Current pharmaceutical options, while effective in managing inflammation, often come with substantial risks that limit their long-term use. The search for novel anti-inflammatory agents, particularly from natural sources like medicinal plants, addresses this critical gap in research. Kadpaayan (Psychotria luzoniensis), known for its traditional use in treating various ailments including headaches and gastrointestinal issues, presents a promising candidate due to its reported medicinal properties. Investigating its anti- inflammatory potential could offer new insights into safer treatment alternatives for inflammation-related disorders.

The primary objective of this study is to evaluate the anti-inflammatory activity of the ethanolic leaf extract of Kadpaayan (Psychotria luzoniensis) using an egg white-induced inflammation model in albino rats. By conducting rigorous experimental analysis, the study aims to assess the extract's efficacy in mitigating inflammation symptoms and explore its potential mechanisms of action. Ultimately, this research aims to contribute valuable scientific data that could support the development of Kadpaayan-based therapies as safer alternatives to conventional anti- inflammatory drugs, particularly in contexts where access to pharmaceutical treatments is limited.

METHODOLOGY

This chapter deals with the method and procedures of the study including the collection and preparation of the plant sample for phytochemical test, and bioassays, research design, data collection, data gathering tool, data gathering procedure and data analysis. These are employed to obtain relevant information needed for the study.

Research Design

This study used the experimental research design to evaluate the anti-inflammatory activity of Kadpaayan (Psychotria luzoniensis) in egg white-induced inflammation in Albino rats.

Procedure

To conduct the research on the anti-inflammatory properties of the ethanolic leaf extract of Kadpaayan (Psychotria luzoniensis), a systematic procedure was followed. Initially, Kadpaayan leaves were collected from Tawi Penablanca, Cagayan, early in the morning to preserve photosensitive components. A total of 15 kg of leaves were harvested and thoroughly cleansed to remove impurities. Subsequently, the leaves underwent air-drying on paper towels for approximately one week to ensure complete dehydration.

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After drying, the extraction of active compounds was performed using 70% ethanol through maceration. The leaves were immersed in ethanol for 72 hours in clear glass containers, following established protocols. The resulting extracts were then subjected to solvent evaporation using a water bath to concentrate the active constituents, ensuring the removal of residual ethanol confirmed by flame tests. Phytochemical analysis was conducted to identify secondary metabolites present in the ethanolic extracts. Standard procedures were employed to detect coumarins, saponins, and phenols, confirming the presence of these phytochemicals in the extract. This qualitative analysis provided insights into the potential bioactive components responsible for anti-inflammatory effects.

For biological testing, thirty albino rats were used to evaluate the anti-inflammatory efficacy. Paw edema was induced by injecting egg white, and the volume displacement method was employed to measure inflammation. The rats were randomly assigned to six treatment groups, including different concentrations of the ethanolic extract and a standard drug control (Diclofenac). The volume of paw edema was measured at regular intervals over five hours post- treatment administration, allowing for the assessment of each treatment's effectiveness in reducing inflammation.

Data Analysis

The researchers used the following statistical tools to treat the data. A two-way ANOVA estimates how a quantitative variable's mean changes over time when the levels of two categorical variables are changed. A two-way analysis variance (ANOVA)a statistical test used in this study to determine the impact of two nominal predictor variables on a continuous outcome variable. A statistical technique, the weighed mean equation, uses the product of the weight and their respective means to determine the average.

RESULT

This chapter presents the data, results and findings of this study which it is presented in tabular forms.

Table 1. **Phytochemical Analysis of Secondary Metabolites in Kadpaayan (*Psychotria luzoniensis*) Leaves Extract.**

Constituent	**Results**
Coumarins	(+)
Saponins	(+)
Phenols	(+)

Phytochemical screening for the selected secondary metabolites were conducted to assess their presence in the crude extract of the plant sample. Table 1 shows the presence of (coumarins, saponins, and phenols) provided the positive results to different specific test reagents. These secondary metabolites accounted for the evaluation of the anti- inflammatory activity of Kadpaayan (Psychotria luzoniensis) in egg white- induced inflammation in albino rats. Therefore, it implies that Kadpaayan has secondary metabolites that has an anti-inflammatory effect in which can help in the study.

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The result is also supported by the study of Calixtro et al., on 2017 that the study findings on phytochemical screening, which found that Rubiaceae family includes Psychotria luzoniensis had anti-inflammatory properties having the presence of coumarins, saponins, flavanoids and phenols. (Calixtro et al., 2017)

Table 2. **Mean Volume Water Displaced Before Induction of Inflammation**

Concentration	**Weighted Mean**
NEGATIVE CONTROL	0.70
100% ETHANOLIC LEAF EXTRACT	0.79
75% ETHANOLIC LEAF EXTRACT	0.84
50% ETHANOLIC LEAF EXTRACT	0.77
25% ETHANOLIC LEAF EXTRACT	0.61
POSITIVE CONTROL	0.49

The Anti-Inflammatory potential of Ethanolic Leaf Extract of Kadpayaan (Psychotria luzoniensis) was investigated, using egg white induce in albino rats that leads to inflammation. The mean volume of water displaced prior to inflammation were assessed in order to identify baseline state before the start of inflammation.

As shown in table, the highest weighted mean is the 75% ethanolic leaf extract, which displaced 0.84 ml, next the 100% ethanolic leaf extract, which displaced 0.79 ml, then negative control, which displaced 0.70 ml, after that the 50% ethanolic leaf extracts, which displaced 0.77 ml and 25% ethanolic leaf extract, which displaced 0.61 ml. The lowest weighted mean is the positive control, which displaced 0.49 ml, indicating that the rats were still free of inflammation.

This implies that the rats were not experiencing any inflammation when the research is conducted.

Table 3. **Mean Volume of water Displaced After Induction of Inflammation.**

TIME	**CONCENTRATION**	**WEIGHTED MEAN**
After induction of egg white Paw Measurement in(ml)	NEGATIVE CONTROL	0.97
	100% ETHANOLIC LEAF EXTRACT	1.14
	75% ETHANOLIC LEAF EXTRACT	0.16
	50% ETHANOLIC LEAF EXTRACT	1.06

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	25% ETHANOLIC LEAF EXTRACT	1.13
	POSITIVE CONTROL	0.49
30mins	NEGATIVE CONTROL	1.11
	100% ETHANOLIC LEAF EXTRACT	1.39
	75% ETHANOLIC LEAF EXTRACT	1.41
	50% ETHANOLIC LEAF EXTRACT	1.35
	25% ETHANOLIC LEAF EXTRACT	1.07
	POSITIVE CONTROL	1.36
60mins	NEGATIVE CONTROL	1.17
	100% ETHANOLIC LEAF EXTRACT	1.34
	75% ETHANOLIC LEAF EXTRACT	1.36
	50% ETHANOLIC LEAF EXTRACT	3.43
	25% ETHANOLIC LEAF EXTRACT	1.33
	POSITIVE CONTROL	1.30
90mins	NEGATIVE CONTROL	1.19
	100% ETHANOLIC LEAF EXTRACT	1.30
	75% ETHANOLIC LEAF EXTRACT	1.33
	50% ETHANOLIC LEAF EXTRACT	1.46
	25% ETHANOLIC LEAF EXTRACT	1.35
	POSITIVE CONTROL	1.26
120mins	NEGATIVE CONTROL	1.23
	100% ETHANOLIC LEAF EXTRACT	1.28

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	75% ETHANOLIC LEAF EXTRACT	1.30
	50% ETHANOLIC LEAF EXTRACT	1.41
	25% ETHANOLIC LEAF EXTRACT	1.34
	POSITIVE CONTROL	1.21
150mins	NEGATIVE CONTROL	1.25
	100% ETHANOLIC LEAF EXTRACT	1.24
	75% ETHANOLIC LEAF EXTRACT	1.27
	50% ETHANOLIC LEAF EXTRACT	1.37
	25% ETHANOLIC LEAF EXTRACT	1.31
	POSITIVE CONTROL	1.14
180mins	NEGATIVE CONTROL	1.27
	100% ETHANOLIC LEAF EXTRACT	1.19
	75% ETHANOLIC LEAF EXTRACT	1.23
	50% ETHANOLIC LEAF EXTRACT	1.32
	25% ETHANOLIC LEAF EXTRACT	1.27
	POSITIVE CONTROL	1.25
210mins	NEGATIVE CONTROL	1.24
	100% ETHANOLIC LEAF EXTRACT	1.13
	75% ETHANOLIC LEAF EXTRACT	1.16
	50% ETHANOLIC LEAF EXTRACT	1.28
	25% ETHANOLIC LEAF EXTRACT	1.23
	POSITIVE CONTROL	0.96

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240mins	NEGATIVE CONTROL	1.22
	100% ETHANOLIC LEAF EXTRACT	1.08
	75% ETHANOLIC LEAF EXTRACT	1.09
	50% ETHANOLIC LEAF EXTRACT	1.19
	25% ETHANOLIC LEAF EXTRACT	0.97
	POSITIVE CONTROL	0.84
270mins	NEGATIVE CONTROL	1.20
	100% ETHANOLIC LEAF EXTRACT	0.94
	75% ETHANOLIC LEAF EXTRACT	1.00
	50% ETHANOLIC LEAF EXTRACT	1.13
	25% ETHANOLIC LEAF EXTRACT	0.99
	POSITIVE CONTROL	0.71
300mins	NEGATIVE CONTROL	1.17
	100% ETHANOLIC LEAF EXTRACT	0.85
	75% ETHANOLIC LEAF EXTRACT	0.91
	50% ETHANOLIC LEAF EXTRACT	1.01
	25% ETHANOLIC LEAF EXTRACT	0.96
	POSITIVE CONTROL	0.57

In this table presented the mean volume of water displaced after the induction of 0.02 ml of egg white that caused inflammation in albino rats at different time points.

One hour after induction of inflammation, the highest weighed mean is the 50% ethanolic leaf extracts, which displaced 3.43 ml, followed by 75% ethanolic leaf extracts, which displaced 1.36 ml, 100% ethanolic leaf extracts, which displaced 1.34 ml, then 25% ethanolic leaf extracts, which displaced 1.33 ml, and the positive control, which displaced 1.30 ml. Lastly, the lowest weighted mean was the negative control, which displaced 1.17 ml. As shown in table, different concentrations of the ethanolic leaf extract showed fluctuations at the subsequent time points, 120, 150-, 180-, minutes post-

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induction while negative control only shown a decrease in paw edema begins at 180- minutes after treatment. However, the positive control displays a moderately anti- inflammatory effect. At 300 minutes post-induction, the negative control and positive control groups continue to display water displacements. The ethanolic leaf extract groups, especially 25%, 100%, and 75%, show progressively lower water displacement values, indicating its effectiveness as an anti-inflammatory.

The findings of this study implies that the ethanolic leaf extract in different concentrations displays anti-inflammatory potential, that is comparable with the positive control (Diclofenac).

It can be supported in the European Scientific Journal (2014) the leaf conducted to evaluate the anti- inflammatory activity, test was done with the used of the different concentrations of the leaf extract, 100%, 75%, 50%, and 25% and were used to treat the proteins in the albumin solution. As a results, at twenty five percent concentration the leaf extract is more effective as an anti-inflammatory than other higher concentrations. So, this implies that the Kadpaayan (Psychotria luzoniensis) in 25% concentration have anti- inflammatory activity, these would attribute based on the results.

Table 4. **Two- Way Anova**

VARIABLES	f – value	p – value	Decision
CONCENTRATIONS	3.66	.003*	**NOT SIGNIFICANT**
ACROSS TIME	5.70	.000*	**SIGNIFICANT**
INTERACTION	1.09	.316	**NOT SIGNIFICANT**

The two-way ANOVA analysis reveals important insights into the study's data. First, the concentration of the ethanolic leaf extract significantly influences the mean volume of water displaced after the induction of inflammation, as indicated by a substantial f-value of 3.66 and a p-value of .003*. This implies that different extract concentrations have a notable impact on the measured responses, suggesting a dose-dependent effect on inflammation reduction. Second, the timing of measurement, represented by the factor ACROSS TIME, also plays a crucial role, with an even more pronounced effect. The high f-value of 5.70 and an extremely low p-value of .000*, this means that the time at which measurements were taken significantly influences the observed changes in water displacement.

However, when assessing the interaction between concentration and time, the analysis finds no significant effect (p-value = .316), indicating that the influence of concentration on water displacement is not significantly altered by the timing of measurement. These results underscore the significance of both concentration and the timing of measurement in understanding the ethanolic leaf extract's impact on inflammation in albino rats.

CONCLUSION

This study had shown that ethanolic leaf extract of Kadpayaan (Psychotria luzoniensis) showed a potentiality of anti-inflammatory activity possible dose-dependent effect on inflammation reduction, with concentrations of 100%, 75%, 50%, and 25%. The 100% and 25% concentration notably exhibit higher displacement and shown as the more effective among different concentrations with weighted mean of 0.85 and 0.96 as shown in the table 03. Two-way ANOVA analysis emphasizes the significant influence of both extract concentration and measurement timing on water displacement. Concentration shows a dose-dependent effect, while timing significantly impacts changes in displacement. In

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developing potential inflammatory drugs for the management and treatment of disease caused by inflammation, the Kadyapaan leaf ethanolic extract may use as newest plant for anti-inflammatory drug delivery systems.

RECOMMENDATIONS

From the findings of the study, in order to provide some scientific basis for the use of leaf extraction of the plants from our environment in the management of pain and some inflammatory activity. The researchers would like to recommend:

1. Future researchers may investigate or explore another plant that has a potential in anti-inflammatory activity to formulate a new system of drugs.

2.Future researchers also may segregate the active constituents from the active extract exhibiting a significant anti-inflammatory activity.

3.Future researchers may consider the use Carageenan powder instead of egg white to cause inflammation.

4.Future researchers would also try to use the other parts of the plants in evaluating the anti-inflammatory activity.

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