International Journal Of Engineering Research And Development
e- ISSN: 2278-067X, p-ISSN: 2278-800X, www.ijerd.com
Volume 15, Issue 2 (February 2019), PP.53-57

The Anti-Inflammatory Activity of Saponin Glycosides Obtained From Musa Paradisiaca Linn. (Cardaba) Young Leaves on Male White Mice

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ABSTRACT: *The study determined the anti–inflammatory activity of saponin glycosides extract obtained from the young leaves of Musa paradisiaca Linn. (cardaba) to reduce the inflamed paw of male white mice. The experimental method was employed in the conduct of the study.*

The fresh young leaves of Musa paradisiaca Linn. (cardaba) were gathered early in the morning. The plant sample was washed and cleaned thoroughly with water to remove the dirt and defected leaves. It was air dried and one hundred grams of comminuted plant sample was weighed. Then, it was macerated with 80% ethyl alcohol for 48 hours and subjected to reflux distillation for an hour and was filtered through Buchner funnel. A portion of filtrate was tested for the presence of saponin glycosides. The froth formation test was used to determine the presence of saponin glycosides in the plant extracts. The filtrate from reflux distillation was added with 10% lead acetate and then it was filtered and passed through hydrogen sulfide in order to isolate and purify the saponin glycoside. A Libermann–Burchard confirmatory test was done to confirm the presence of saponin glycosides in the extract. The isolated and purified extract was evaporated to syrupy consistency. Then, the extract was ready for administration.

A total of twenty–one male white mice were utilized in three groups (seven male white mice in each group) with weights' ranging from 18–30 grams was used for this assay method. The twenty–one male white mice were grouped into three, designating them as first group for experimental control, second group for positive control, and third group for negative control. The groupings was done randomly using a drawn by lot method. The first group received the Cardaba leaf extract, the second group received Indomethacin and the third group received 0.9% Normal Saline Solution.

The left hind paw was measured using the plethysmometer. A 1% carrageenan suspension was used to induce inflammation to the paw of male white mice. After an hour of induction, the paw was again measured. Each group was given corresponding treatment. The inflammation of the left hind paw was again measured and recorded every hour for six hours.

Based on the results of the study, the experimental group had 112.24% mean reduction in paw volume compared to the positive control group with 135.71% mean reduction in paw volume. This concluded that the saponin glycoside obtained from the young leaves of Musa paradisiaca Linn. (cardaba) had an anti–inflammatory activity that was comparable with the positive control group which was the indomethacin.

KEYWORDS:- *Anti-inflammatory activity, Saponin glycosides, Musa Paradisiaca Linn.*

Date of Submission: 30-06-2019 Date of acceptance: 19-07-2019

I. INTRODUCTION

Inflammation is the body's attempt at self-protection; the aim being to remove harmful stimuli, including damaged cells, irritants, or pathogens - and begin the healing process (Nordqvist, 2012). This is a symptom or sign of pain which is very common to everyone.

The researcher is focused on treating inflammation with the use of the leaf extracts of a banana plant since the components of the plant have a more possible counteraction with inflammation. This study is aimed to maximize the health benefits of the banana plant. Using its leaves as an alternative anti–inflammatory treatment could give promising results and new benefit from the plant. The researcher is reluctant in the study of synthesizing alternative medicines from herbal plants specifically with the banana plant due to its availability in our country.

The researcher is aiming for the saponin extracts of the banana leaves to have an anti–inflammatory efficacy which is said to have good healing properties against swelling and inflammation (Puakhleem, 2012). The knowledge has motivated the researchers to conduct studies and research in utilization of this plant which are freely available and acceptable to the broad masses of the Filipino people. The formulation of anti-inflammatory syrup from the dried banana leaves may go a long way in providing a quick and natural relief for inflammation thus paving a great contribution to alternative medication.

II. STATEMENT OF THE PROBLEM

This study aims to determine the anti-inflammatory activity of saponin glycosides present in the young leaves of Musa paradisiaca Linn. (Cardaba).

This study will give answers to the following specific problem:

1. What is the mean volume (mL) of the paw of the white male mice before and after induction of inflammation, and after treatment to:

1.1. Positive Control (Indomethacin)
1.2. Experimental Control (Cardaba Extract)
1.3. Negative Control (0.9% Normal Saline Solution)

2. What is the mean reduction of inflammation in volume (mL) of the paw of the male white mice every hour for six hours?
3. What is the mean percent reduction of inflammation of the three different groups?
4. Is there any significant difference in the mean percent reduction of inflammation between the three different groups?

III. METHODOLOGY

This study utilized the experimental method to determine the anti-inflammatory effect of saponin glycosides from the extracts of Musa paradisiaca Linn. (Cardaba) leaves on the paw of male white mice with carageenan-induced swelling.

Twenty-one male white mice were used as the test animals in this experiment with preferable weights' ranging from 18-30 grams. It was carefully monitored and was provided with pellets and water to sustain the needs and to prevent drastic changes in behavior. The male white mice were placed in a cage and acclimatized for one week prior to the experimentation. The mice were group randomly into three using draw by lot method, designating them as first group for Experimental Control which received saponin glycosides extract, the second group for Positive Control which received Indomethacin and the last group for Negative Control which received 0.9% Normal Saline Solution. A plethysmometer was used to measure the mean paw volume of the mice before and after the induction of carrageenan for inflammation, and after the treatment of the three different groups. The data obtained in the experimentation was recorded in the observation sheet.

The fresh young leaves of cardaba were gathered early in the morning when the photosynthesis is most active. The plant sample was washed and cleaned thoroughly with water to remove the dirt and defected leaves. It was air dried and one hundred grams of comminuted plant sample was weighed. Then, it was macerated with 80% ethyl alcohol for 48 hours and subjected to reflux distillation for one hour and was filtered though Buchner funnel. The filtrate was evaporated to pilular consistency (Guevarra, 2005). A portion of the pilular extract was tested for the presence of saponin glycosides.

The froth formation test was used to determine the presence of saponin glycosides in the plant extracts. The alcohol extract was mixed with 2mL of 10% Gogo extract, followed by the addition of 10mL distilled water. It was shaken vigorously for 30 seconds and allowed to stand over a period of 30 minutes. A froth which appears on standing indicates the presence of saponin glycosides (Guevarra, 2005).

A 40mL solution was prepared in distilled water using 20g of the dried Musa paradisiacal Linn. (cardava) young leaves. This was extracted twice with 20mL diethyl ether and shaken vigorously. The diethyl ether was discarded and the retained aqueous layer was further extracted with 60mL n-butanol. The combined n-butanol extracts were washed twice with 10mL of 5% NaCl. The washed extract was evaporated to pilular consistency (Shashankk, Suresh, 2013). A 100mg carrageenan sodium salt was weighed and sprinkled on a 10mL sodium chloride, NaCl (0.9%w/v). It was to soak for an hour and stirred to suspend the mixture (Guevarra, 2005). A 200mg of Indomethacin capsule was diluted with 200mL 0.9% Normal Saline Solution (1mg/1mL) (Gahart and Nazareno, 2013).

The mice were weighed individually prior to induction of inflammation and administration of different groups. The left hind paw of the male white mice was measured using Plethysmometer prior to induction. Acute inflammation was induced into the plantar surface of the left hind paw of mice with 0.1mL of 1% carrageenan suspension. The left hind paw of the mice was again measured after an hour of induction (Guevarra, 2005).

The test solutions were administered orally using a gastric gavage. The dose of Indomethacin is computed based on the weight of the mice using adult dose of Indomethacin (200mg) against the weight of the mice and standard weight. The dose of the isolated saponin glycosides extract was 200mg/kg and the dose of 0.9% Normal Saline Solution was 0.3mL since it is tolerable amount the mice can take per oral dose. This was done by using a tuberculine syringe without needle through a gastric gavage and there is no standard dosage.

The inflammation of the left hind paw was again measured and recorded every hour for six hours (Guevarra, 2005).

The percent reduction formula was used to determine the efficacy of the test solutions (Guevarra, 2005).

Test for equal variance was used as a requirement to be able to conduct One-Way Analysis of Variance (ANOVA). ANOVA was performed to determine if there is a significant result, Pasthoc: Bonferroni-Herroni will be conducted. It is used since the study will only be utilizing a small sample size.

IV. PRESENTATION, ANALYSIS; AND INTERPRETATION OF DATA

The study was conducted for the anti-inflammatory activity of saponin glycosides obtained from Musa paradisiaca Linn. (Cardaba) young leaves through experimental method. Tables are presented to show the results of the data gathered.

Table 1. The Mean Volume (mL) of the paw of the male white mice before and after induction of inflammation, and after treatment.

Paw Volume (mL)								
Test Groups	**Induction of Inflammation (mL)**		**Hours Interval**					
	Before	**After**	**1**	**2**	**3**	**4**	**5**	**6**
Experimental Control (Plant Extract)	0.03	0.0800	0.0800	0.0686	0.0543	0.0429	0.0300	0.0271
Positive Control (Indomethacin)	0.0343	0.0814	0.0814	0.0729	0.0600	0.0442	0.0243	0.0243
Negative Control (0.9%NSS)	0.0314	0.0814	0.0814	0.0814	0.0814	0.0800	0.0757	0.0728

The table presents the mean volume (mL) of the paw of the male white mice before and after induction of the 1% carrageenan suspension. The baseline is the mean volume before inflammation. A decrease in the paw volume showed after induction of the inflammation. This was monitored every hour for six hours. This showed that the reduction of inflammation in the experimental control group is comparable with the positive control group in the fifth hour wherein the mean paw volume has returned to its baseline.

Table 2. The Mean Reduction of Inflammation in Volume (mL) of the paw of the male white mice every hour for six hours.

Test Groups	**Paw Volume (mL)**					
	Hours Interval					
	1	**2**	**3**	**4**	**5**	**6**
Experimental Control (Plant Extract)	0	0.0114	0.0257	0.0371	0.0500	0.0529
Positive Control (Indomethacin)	0	0.0085	0.0214	0.0372	0.0571	0.0571
Negative Control (0.9%NSS)	0	0	0	0.0014	0.0057	0.0086

The table presents the mean reduction of inflammation of the paw of the male white mice between the three different control groups after the administration of the test solutions. It shows that the experimental group is comparable to the positive group in reduction of inflammation in the third hour up to the succeeding hours.

Table 3. The Mean Percent Reduction of Inflammation between the Positive, Negative and the Experimental Group

Test Groups	Paw Volume (mL)					
	Hours Interval					
	1	2	3	4	5	6
Experimental Control (Plant Extract)	0	22.8	51.4	74.2	100	100
Positive Control (Indomethacin)	0	18.05	45.44	78.98	100	100
Negative Control (0.09% NSS)	0	0	0	2.8	11.4	17.2

The table shows the increase in the mean paw volume of the male white mice in the three control groups were the same after induction of inflammation. The experimental group and the positive group showed

the decrease in the second hour but in the statistical analysis, both groups are significant in the third hour. The positive control group has a greater mean percent reduction of inflammation in the fifth hour than the experimental group. The negative group did not showed any reduction in the first to third hour but it slightly decreased in the following hours which means 0.9%NSS has no anti-inflammatory activity. Although the positive group has a greater mean percent reduction of inflammation, the experimental group (saponin glycoside extract) still has a potential anti-inflammatory activity.

Table 2.3 One way ANOVA table comparing decreases in inflammation within six hours observation with Post Hoc (LSD)

	POS n=7		EXP n=7		NEG n=7						Post Hoc
	$\bar{X}$	*sd*	$\bar{X}$	*sd*	$\bar{X}$	*sd*	F	p (.05)	Decision	Interpretation	Bonferroni
hr_1	0	0	0	0	0	0	0.048	0.953	Do not reject Ho	Not Significant	
hr_2	21.42857	24.93377	22.17687	23.07904	0	0	1.380	0.277	Do not reject Ho	Not Significant	
hr_3	52.38095	41.49177	49.89796	17.27564	0	0	6.689	0.007	Reject Ho	Significant	1=2: 2≠3: 1≠3
hr_4	92.85714	55.1573	76.06803	13.64067	2.857143	7.559289	22.193	0.000	Reject Ho	Significant	1=2: 2≠3: 1≠3
hr_5	135.7143	55.63486	105.8163	24.12562	11.57143	11.69065	51.529	0.000	Reject Ho	Significant	1=2: 2≠3: 1≠3
hr_6	135.7143	55.63486	112.2449	26.31387	20	21.16951	41.468	0.000	Reject Ho	Significant	1=2: 2≠3: 1≠3

Notes: 1 = pos, 2 = exp, 3 = neg; ≠ significant difference ; = not significant

Significant at the .05 level of significance (p<.05)

The one way analysis variance or ANOVA is a technique used to compare means of two or more samples (using the F distribution). Using the ANOVA table, results can easily be determined from the three groups (positive, experimental, negative). The f and p values are the two main bases to consider whether the values are of significance or not. In this table, the values from the first to the second hour are not significant since the three groups have not yet taken effect. The third hour however, showed significant values therefore we can determine that an effect has already begun in the two groups which are the positive and the experimental groups. Up to the sixth hour the values continued to be significant and showed a difference of a decrease in the inflammation between the three groups. From this, we could conclude that the positive control showed the most anti – inflammatory effect as expected while our experimental control also gave anti-inflammatory effect comparable to that of the positive control. The negative control did not give a decrease in the inflammation.

V. SUMMARY OF FINDINGS

Based on the data gathered, the results showed that saponin glycosides obtained from Musa paradisiaca Linn. (cardaba) young leaves exerts an anti-inflammatory activity as it showed a reduction of inflammation on the paw of the male white mice in the third hour of monitoring, the mean reduction of inflammation was then used for the computation of the mean percent reduction of the inflammation of the extracts and control. In the first two hours, there is no significant difference in the computed f-value of the positive and experimental group but in the third to sixth hour, both groups have a significant difference. This means that although the positive control (indomethacin) has a greater possibility in mean percent reduction of inflammation because it is an anti-inflammatory drug, the experimental group (saponin glycosides extract) still has a potential in anti-inflammatory activity. The Analysis of Varians (one-way) indicated that there is no significant difference between the positive and the experimental group and thus the saponin glycosides is comparable and is as effective as the positive control (indomethacin), statistically.

VI. CONCLUSIONS

Based on the results obtained from the actual experimentation, the researcher concluded that the saponin glycosides obtained from the young leaves of Musa paradisiaca Linn. (cardaba) has an anti-inflammatory activity that is comparable from the effect of indomethacin, since the experimental control and positive control manifested 100% mean reduction in paw volume.

VII. RECOMMENDATIONS

Based on the findings and conclusion of the study, the following recommendations are forwarded: More scientific based research on the anti-inflammatory activity of saponin glycosides obtained from Musa paradisiaca Linn. (cardaba) young leaves to conduct another therapeutic activities of saponin such as analgesic, anti-hypertensive, anti-microbial activity, and wound healing. More scientific based research on the anti-inflammatory activity of saponin glycosides from Musa paradisiacal Linn. (cardaba) young leaves to conduct the adverse drug effect activity. Pharmaceutical industries are encouraged to convert the saponin glycosides obtaind from Musa paradisiaca Linn. (cardaba) young leaves into its applicable pharmaceutical dosage form, subject to formulation to its shelf-life studies and conduct necessary parameters or test to determine potency and bioavailability. More studies are needed in animals and humans on the kinetics of Musa paradisiaca Linn (cardaba) young leaves and its constituents and on the effect of consumption over a long period of time.

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Edgardo S. Geniston" The Anti-Inflammatory Activity of Saponin Glycosides Obtained From Musa Paradisiaca Linn. (Cardaba) Young Leaves on Male White Mice" International Journal Of Engineering Research And Development , vol. 15, no. 2, 2019, pp 53-57