
Edema and Granuloma Inhibiting Potential of Banana (*Musa Paradisiaca* Linn.), Guava (*Psidium Guajava* Linn.) and Lima Bean (*Phaseolus Lunatus*) Leaf Extracts on Acute and Chronic Models of Inflammation

Leah S. Guzman*, Dvm, Mvst, Phd¹ and Celso C. Garcia, Jr.²

^{1,2}School of Veterinary Medicine, Isabela State University, Echague Campus, Philippines

Leah S. Guzman, Dvm, Mvst, and Celso C. Garcia, Jr. (2016). Edema And Granuloma Inhibiting Potential Of Banana (*Musa Paradisiaca* Linn.), Guava (*Psidium Guajava* Linn.) And Lima Bean (*Phaseolus Lunatus*) Leaf Extracts On Acute And Chronic Models Of Inflammation. International Journal of Agricultural Technology 12(7.1):1375-1384.

The study was primarily conducted to determine the edema and granuloma inhibiting potential of banana, guava and lima bean leaf extracts on acute and chronic models of inflammation in male and female guinea pigs.

A total of sixty three (63) 2-3 month old apparently healthy male and female guinea pigs, weighing 180-200 grams were distributed to four treatments, three replicates consisting of three animals per replicate. The four treatments are: Saline (Treatment 1), Banana (Treatment 2), Guava (Treatment 3) and Lima Bean (Treatment 4) leaf extracts. Patch and scratch methods were used in evaluating the effects of the test materials on acute inflammatory conditions 24 hours and 72 hours post-treatment while induced pellet granuloma method was used for evaluating the effects of the test materials on chronic inflammatory conditions. All data gathered were statistically analyzed using ANOVA, LSD and Frequency distribution.

Lima bean provided the most recorded anti-edema (9/9) agent indicated by a score of 0 (No edema) using the Patch Test 72 hours post-treatment. Lima bean provided one edema score classified as moderate to severe. Scores of 1 and 2 edema types were also reduced using banana (8/9) and guava (8/9). Statistical analysis of the patch test however provided no significant difference between guava and banana but both treatments were found to be significantly different with lima bean and saline as anti-edema agent.

Guava (9/9) 24 and 72 hours post-treatment provided the most effective anti-edema respectively using the Scratch Test Method. Guava was efficient to moderate to severe type of edema. Banana and lima bean also provided a 100% anti-edema effect for slight to moderate to severe types of edema 72 hours post-treatment. Effectiveness of the plant materials as anti-edema agent revealed no significant differences but statistically different with the control saline. Results also revealed a non-irritating effect based on PI of 0.08 and 0.0 for banana, guava and lima bean respectively.

Banana (1.55 mg) provided the least granuloma formation followed by guava (1.67 mg), mefenamic acid (1.56 mg) lima bean (2.00 mg) and saline (2.67 mg). Statistical analysis, however, provided no significant effect of the said treatments in granuloma formation.

The observed results indicate that most of the plant materials were effective only for acute inflammatory conditions but not on chronic cases.

Keywords: Edema, granuloma, acute and chronic inflammation

*Corresponding Author: Leah S. Guzman; Email: namzughuels03276@gmail.com

Introduction

Inflammatory conditions may be considered acute and chronic and based on visual observation of the ancients is characterized by five cardinal signs, namely redness (*rubor*), swelling (*tumour*), heat (*calor*), pain (*dolor*) and loss of function (*functio laesa*). Although, in ancient times inflammation was recognized as being part of the healing process, up to the end of the 19th century, inflammation was viewed as being an undesirable response that was harmful to the host (Cheville 1999, Tizzard 2004, Guyton and Hall, 2006). Although, the classical description of inflammation accounts for the said visual changes seen, majority of the loss of function is often due to oedema and pain. Oedema is the result of increased passage of fluid from dilated and permeable blood vessels into the surrounding tissues, infiltration of cells into the damaged area, and in prolonged inflammatory responses deposition of connective tissue (Punchard *et al.*, 2004).

To alleviate the condition, majority of synthetically prepared anti-inflammatory drugs were used. Readily available drugs however in the market creates few adverse effects hence, suggests the need of a new and safe treatment. The use of alternative natural products possibly from plant sources with no recorded adverse effects could be a solution. According to WHO, 70% to 80% of the population in many developed countries have used some form of alternative or complementary medicine which traditionally were plant products. Because of their chemical diversity, natural products coming from plant serves as rich source for discovery of potential drugs for inflammation.

There are hundreds of phytoconstituents reported to have many pharmacological activities and most of these reports are of academic interest but very few find entry in clinical trials (Kumar *et al.*, 2013). Three (3) EVB-M materials to include banana, guava and lima bean for instance was proven safe by the people of Echague Isabela Philippines in improving skin diseases (Guzman, 2015) however, the extent on their use for acute and chronic inflammatory conditions needs yet to be investigated, hence, this study.

Materials and methods

Authentication, Collection and Preparation of Plant Materials

All plants that were used in the study were taxonomically identified and authenticated at the Botany Division Office, Philippine National Museum, Padre Burgos Drive, Manila City, 1000, Metro Manila.

Fresh mature leaves of guava, banana and lima bean were collected at the Municipality of Echague, Isabela. Two hundred grams (200g) of the fresh plant material were finely cut and were submerged in 300 ml of 95%

alcohol, stoppered for 24 hours and filtered and the collected filtrates were distilled through distillation process, the collected distillate were the final products used for the analysis.

IACUC Certification and Management of the Test Animals

Before the purchase and use of the test animals for experiment proper, IACUC certification (Reference no. AR-2015-186) was first acquired at the Bureau of Animal Industry.

A total of sixty three (63) 2-3 month old healthy male and female guinea pigs, weighing 180-200g served as the experimental animals of the study. Experimental animals were acclimatized for 8 days before the start of the study and maintained in laboratory environment, fed with standard chow pellet diet with water given free access to animal. At the start of the experiment, all experimental animals were weighed and kept in an individual observation cages. All animals were fasted from food and water 10 hours before each test.

Experiment Proper

Patch Test

The skin lateral to the spinal groove were shaved and cleaned. The left sides of the groove were utilized as the negative control site and the right side as the test drug site. The sites were cleaned with 70% alcohol to make a patch incision (approximately 1 cm in diameter) using scalpel blade. The test drug and negative control on the inoculation sites were given drops appropriately. Sites were covered with sterilized gauze (1x1 cm in size) kept in place by the use of surgical tape. The test animal was left for 24-72 hours during the time from which they rendered immobile.

Patches were removed after 24 hours exposure and reactions were evaluated according to the scores in Table 1. Another reading or scoring was done after 72 hours. Average scores of the 24 and 72 readings were computed.

Scratch Test

The procedure and scoring method was the same as in patch test but with a light modification. The skin of the test animal were abraded by lightly scratching the skin five to seven times with a gauge of hypodermic needle, followed by application of the test drug and the saline solution (negative control).

Test Scoring

Table 1. Evaluation of Skin Reaction through Edema Formation.

Edema formation	Score
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area will defined by definite raising)	2
Moderate edema (raised approximate 1 millimeter)	3
Severe edema raised more than 1 millimeter and extending beyond the areas of exposure)	4
Total Edema Score	4

The averages of the scores for patch and scratch test was combined. This combined average is referred to as primary irritation index.

Table 2. Primary Irritation Index (Classification System).

PII	Classification
<0.5	Non-irritating
0.5 – 2.0	Slight irritating
2.1 – 5.0	Moderate irritating
> 5.0	Severe irritating

Induced Cotton Pellet Granuloma Formation Method

Two mg sterile cotton pellets (cut from absorbent cotton rolls) were implanted subcutaneously on each nape of mice, and the test drug was administered 3 times daily for 7 days. Animals were sacrificed 24 hours after the final application. The cotton pellet was dissected out together with the surrounding granuloma. The dissected pellet was dried and weighed to get the actual granuloma formed.

The above procedure was repeated using a negative control (saline solution) and a positive control (a standard anti-inflammatory drug/mefenamic acid).

Analysis of Data

All data collected were subjected to ANOVA and subsequently to LSD (Least Significant Differences) for the determination of the differences between means. Frequency distribution of each scores was also determine for each test.

Experimental Design

The study utilized a Complete Randomized Design (CRD) with each treatment consisting of 9 animals. The four treatments consisted of 3

replicates, with 3 animals each replicate. All test animals were distributed randomly in the following treatment: Patch and Scratch test (Treatment 1 – Saline, Treatment 2 – Banana, Treatment 3 – Guava, Treatment 4 – Lima Bean) and Induce Granuloma Formation (Treatment 1 – Mefenamic Acid (positive control), Treatment 2 – Saline (negative control), Treatment 3 – Banana, and Treatment 4 – Guava and Treatment 5 – Lima Bean).

Results and Discussion

The anti-inflammatory effect of banana, guava and lima bean were evaluated using the Patch and Scratch Method. Patch and Scratch method was used for evaluating the effects of the test materials in acute inflammatory conditions based on edema and primary irritation index 24 to 72 hours post-treatment.

Edema Formation Using the Patch Method

Table 3 below provides the evaluated skin reactions through edema formation after 24 and 72 hours following application of the three extracts. Results revealed that banana (7/8), guava (8/8), and lima bean (8/8) provided a score of 0 (no edema), whereas the control group obtained the highest score of 1 (slight edema/ barely perceptible edema) 24 hours post-treatment. Majority of the results provided the same score of 0 (no edema) for banana (8/9), guava (8/9) and lima bean (9/9) after 72 hours.

Although all of the three plant materials provided an effective result as reflected from the majority scores of 0 (no edema), results still showed that lima bean is the best treatment providing a 100% recovery of the treated animals from edema formation.

Regardless of the number of animals treated, banana and guava provided only one type of edema score (i.e. score of 1/ very slight edema) after 24 hours, while the control group provided two edema score types with score of 1 (very slight edema) and 2 (slight edema) both for 24 hours and 72 hours. Average scores after 24 to 72 hours provided the same edema effect of 0.16 for the three plant materials all of which falls under the score of 0 (no edema) while majority of the control group obtained a score of 1 (very slight edema).

Table 3. Formation of Edematous Lesions 24 and 72 Hours Post-treatment of saline , Banana, Guava and Lima Bean Leaf Extracts Using Patch Test Method

Treatment	Hours of observation	No. of experimental animals	Scores of formation(frequency) of edema					Total edema Score Types	Average score after 24 & 72 hours	Edema score Indication
			0(%)	1(%)	2(%)	3(%)	4(%)			
Treatment 1 Saline	24	9	0 (0)	5 (55)	1 (11)	0 (0)	0 (0)	2	0.83 a	Barely perceptible
	72	9	1 (11)	1 (11)	1 (11)	2 (22)	0 (0)	3		
Treatment 2 Banana	24	9	7 (77)	2 (22)	0 (0)	0 (0)	0 (0)	1	.16b	No edema
	72	9	8 (88)	1 (11)	0 (0)	0 (0)	0 (0)	1		
Treatment 3 Guava	24	9	8 (88)	1 (11)	0 (0)	0 (0)	0 (0)	1	0.16 b	No edema
	72	9	8 (88)	1 (11)	0 (0)	0 (0)	0 (0)	1		
Treatment 4 Lima bean	24	9	8 (88)	0 (0)	0 (0)	1 (11)	0 (0)	1	0.16 a	No edema
	72	9	9 (99)	0 (0)	0 (0)	0 (0)	0 (0)	1		

Note; Score 0=No edema; 1=Very slight edema; 2=Slight edema; 3=Moderate edema; 4=Severe edema

Means with the same letter are not significantly different;

Ns – Not significant

Statistical analysis revealed no significant differences between banana and guava but both treatments are significantly lower with saline and lima bean. Such analysis implies that banana and guava have a better effect in reducing edema condition in the test animals.

Edema Formation Using the Scratch Method**Table 4.** Formation of Edematous Lesions 24 and 72 Hours Post-treatment of Saline, Banana, Guava and Lima Bean Leaf Extracts Using Scratch Test Method

Treatment	Hours of observation	No. of experimental animals	Scores of edema formation(frequency)					Total edema Score Type	Ave. score after 24 & 72 hours	Edema score Indication
			0(%)	1(%)	2(%)	3(%)	4(%)			
Treatment 1 Saline	24	9	5 (55)	4 (44)	0 (0)	0 (0)	0 (0)	2	0.33	No edema
	72	9	7 (77)	2 (22)	0 (0)	0 (0)	0 (0)	1		
Treatment 2 Banana	24	9	7 (77)	2 (22)	0 (0)	0 (0)	0 (0)	1	0.00	No edema
	72	9	9 (99)	0 (0)	0 (0)	0 (0)	0 (0)	0		
Treatment 3 Guava	24	9	9 (99)	0 (0)	0 (0)	0 (0)	0 (0)	0	0.00	No edema
	72	9	9 (99)	0 (0)	0 (0)	0 (0)	0 (0)	0		
Treatment 4 Lima bean	24	9	8 (88)	0 (0)	0 (0)	1 (11)	0 (0)	1	0.00	No edema
	72	9	9 (99)	0 (0)	0 (0)	0 (0)	0 (0)	0		

Note; Score 0=No edema; 1=Very slight edema; 2=Slight edema; 3=Moderate edema; 4=Severe edema

**significant at 1% level

Table 4 above provides the evaluated skin reactions for edema formation using the scratch test after 24 and 72 hours following application of the three extracts. A score of 0 (no edema) is the most reading obtained in banana (7/9), guava (9/9), lima bean (8/9) and saline (5/9) after 24 hours. A marked absence of edema was observed for all the plant treatments after 72 hours (9/9) except for the control group having a score of 1 (very slight edema). Such result indicates that three test materials are efficient in treating edema cases 72 hours post-treatment.

Total edema score provided no edema conditions 24 to 72 hours for guava and slight edema after 24 hours for banana and lima bean while saline obtained a slight edema both at 24 and 72 hours. Result clearly shows that banana is the best anti-edema agent at 24 and 72 hours post-treatment.

Average edema results for edema formation using the scratch test provided a score of zero for all the plant material used. Although, majority of the results provided a 90-100% effectively for edema, statistical analysis revealed no significant effects of the test materials.

Table 5. Calculated Edema Primary Irritation of Index in Saline, Banana, Guava and Lima Bean Treated Animals 24 and 72 Hours Post-treatment

Treatment	Method	Hours		PII	Edema score Indication
		24	72		
Treatment Saline	1 Patch Test	8	13	0.58a	Slight irritating
	Scratch Test	4	2		
Treatment Banana	2 Patch Test	2	1	0.08b	Non irritating
	Scratch Test	0	0		
Treatment Guava	3 Patch Test	1	2	0.08b	Non irritating
	Scratch Test	0	0		
Treatment Lima Bean	4 Patch Test	3	0	0.0b	Non irritating
	Scratch Test	1	0		

Note; PII indication <0.5=Non irritating; 0.5-2.0=Slight irritating; 2.1-5.0=Moderate erythema; >5.0=Severe irritating

Means with the same letter are not significantly different;

**significant at 1% level

In terms of the irritating effects to cause edema formation of the test materials (Table 5), results revealed that all of the three plant materials were non-irritating with 0.08 and 0.0 primary irritation index for banana, guava and lima bean respectively. The control group, however, still provided a slight irritating effect. Statistical analysis revealed that the three test materials are non-irritating agents in terms of edema formation.

Table 6. Mean and Actual Granuloma Formation after 7 Days Topical Application of the Test Materials Using the Pellet-Induced Granuloma Formation (IGF)

Treatment	Original pellet weight (mg)	Average weight after 7 days (mg)	Actual Granuloma formed (mg)
Treatment 1			
Mefenamic Acid	2	3.56	1.56
Treatment 2			
Saline	2	4.67	2.67
Treatment 3			
Banana	2	3.55	1.55
Treatment 4			
Guava	2	3.67	1.67
Treatment 5			
Lima Bean	2	4.00	2.00

**significant at 1% level

Table 6 above presents the granuloma formation effect of the test materials after 7 days of pellet implantation. Results showed that the group treated with banana extract has the least granuloma formation with 1.56 increased from the original implanted 2 mg pellet, followed by guava (1.67), mefenamic acid (1.56), lima bean (2.00) and saline (control). The 1.55 mg increase granuloma formation in banana provided an almost equal amount of granuloma formation in the positive control drug mefenamic acid (1.56). Such indicates that the effect of banana for granuloma formation is almost the same with the standard drug. Statistical analysis however provided no significant effect of the said treatments in eliminating granuloma formation.

Analysis indicates that the plant materials are effective only for varying degrees of acute inflammation as indicated in the patch and scratch test results but not for chronic conditions of inflammation identified in granuloma test.

Conclusion and Recommendation

Based on the primary irritation index result, all of the plant materials were found to be non-irritating. Effectiveness of the plant materials was supported by the no significant differences implicating no variation in the treatment of the test materials but statistically different with the control saline. Hence, the effectiveness of the lima bean for edema cases could possibly be more pronounced due added covering applied in the wound in the patch test while all the plant materials were found effective for scratch test method.

Conclusively, lima bean is the most effective anti-edema agent in hygienically protected wound types of slight to moderate to severe as indicated in the Patch Test while most effective anti-edema for the scratch test method was identified in banana and guava respectively. Observed

results indicate that most of the plant materials are effective for acute inflammatory conditions.

From the above conclusions, the following are recommended: (1) The plant materials could be adapted or used in acute inflammatory cases in the absence of a standard veterinary drug; (2) A more accurate and measurable procedure evaluating edema and granuloma formation is recommended and (3) It is suggested that further studies be conducted with the inclusion of a positive control (standard drug) for comparison.

Aknowledgement

The authors gratefully acknowledge the financial support extended by the University Research Department, Isabela State University, Echague Campus, Philippines.

Literature Cited

- Cheville N.F. (1999). Introduction to Veterinary Pathology, 2nd edition. Pp. 135-146
- Guyton, A.C. and Hall, J.E., 2006. Textbook of Medical Physiology 11th edition Elsevier Inc. 19103-2899 Pp.434-436
- Guzman, LS. (2015). Non-experimental Validation of the Effectiveness of Ethno-Veterinary Botanical Medicine (EVB-M) Materials Used in the Municipality of Echague Isabela. *Journal of Agricultural Technology* 11(8): 2377-2389
- Kumar, S., Bajwa B.S., Singh Kuldeep and Kalia A.N., (2013). Anti-inflammatory Activity of herbal plants: a Review. *IJAPBC-Vol2* (2), April-June, 2013. Pp. 274-281 (Punchard et al., 2004).
- Punchard, N.A., Whelani, C.J., and Adcock, I., (2004). The journal of inflammation doi:10.1186/1476-9255-1-1.<http://www.journal-inflammation.com/content/1/1/1>. Accessed 14 August 2015.
- Tizzard, I.R., (2004). *Veterinary Immunology: an Introduction*. 7th Ed. Pp. 19-22 Saunders