ANTI-INFLAMMATORY ACTIVITY OF SAMPA-SAMPALUKAN *(Phyllantus amarus)* AQUEOUS EXTRACT IN WISTAR RATS (*Rattus norvegicus)*

Jackie Lou M. Caballero, Leigh Kasandra T. Capili, Diana Joyce C. Lopez, Roangelie T. Pamittan, Rechel A. Parmis, Allisteir Rodriguez, Gasat V. Umayam Jr.

ABSTRACT

The use of non-steroidal anti-inflammatory drugs in the treatment of diseases associated with inflammatory reactions is fraught with much adverse effect which then poses a major problem in their clinical use. Since herbal drugs gained importance in recent years, the World Health Organization promotes traditional herbal remedies. One of it is *Phyllantus amarus* a world renowned botanical which has been used since ages because of its rich medical value and ethnomedical importance. This study investigates the activity of the aqueous extract of Sampa-sampalukan (*Phyllantus amarus*) plant on its action as anti-inflammatory in male wistarrats carrageenan induced paw edema. Specifically, it aimed to determine the constituent responsible for the anti-inflammatory activity of *Phyllantus amarus* plant, and its efficacy compared to the standard drug, diclofenac sodium. The result of the present study confirmed that *Phyllantus amarus* contains alkaloids that is responsible for the anti-inflammatory activity and showed that there is no significant difference between the positive control, diclofenac sodium, and the different doses of 200mg/kg, 300mg/kg, 500mg/kg, and 1500mg/kg of *Phyllantus amarus* plant extract. Furthermore, statistical analysis using one way analysis of variance (ANOVA) showed that the aqueous extract of *Phyllantus amarus* plants are comparable with the standard drug, diclofenac sodium. Based on the data gathered, the researchers conclude that *Phyllantus amarus* is an effective anti-inflammatory agent.

Key words: *Phyllantus amarus, carrageenan, anti-inflammatory activity, diclofenac sodium, aqueous extract*

INTRODUCTION

Inflammation is a local response of living mammalian tissues to injury. It is body defense reaction in order to eliminate or limit the spread of injurious agent. (Mahat &Patil, 2007) Inflammation is a normal, protective response to issue injury caused by physical trauma, noxious chemicals, or microbiological agents. Inflammation is the body's effort to inactive or destroys invading organisms, remove irritants, and set the stage for tissue repair (Whalen, 2015). The ability to mount an inflammatory response is essential for survival in the fate of environmental pathogens and injury; in some situations and diseases, the inflammatory response may be exaggerated and sustained without apparent benefit and even with severe adverse consequences (Brunton, Parker, Blumenthal, & Buxton, 2008). During

inflammation stimulation of the neutrophil membranes produces oxygen-derived free radicals (Borazan & Furst, 2016)

The World Health Organization (WHO) clearly recognizes these facts and the importance of medicinal plants, hence proposed their authentication the world over. Gastritis can be caused by irritation due to excessive alcohol use, chronic vomiting, stress, or the use of certain medications such as aspirin or other anti-inflammatory drugs.

The use of non-steroidal anti-inflammatory drugs in the treatment of diseases associated with inflammatory reactions is fraught with many adverse effects which then pose a major problem in their clinical use (Adedapo & Ofuegbe, 2013). Since herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness (Sen & Batra, 2013). The World Health Organization currently encourages, recommends and promotes traditional herbal remedies as such drugs are easily available in low cost, are comparatively safe and the people have faith in such remedies (Mohammed, 2008).

Therefore, this study was conducted to assess the anti-inflammatory properties of the whole plant extract of *Phyllantus amarus* in Wistar Rats with a view to provide scientific justification for the researches and traditional claim and the use of *Phyllantus amarus* as a better treatment for anti-inflammatory disease with lesser adverse effect.

Research Questions

Generally, this study aimed to evaluate the anti-inflammatory activity of the Sampa-sampalukan (*Phyllantus amarus*) plant aqueous extract in Male Wistar rats.

Specifically, this study aimed to discuss and answer the following questions:

1. What is the degree of paw edema/ inflammation as evidenced by the volume of water displacement of the different treatment groups?
    a. Baseline
    b. Post-induction of Carrageenan
    c. 1 hour post-treatment
    d. 2 hours post treatment
    e. 3 hours post treatment
    f. 4 hours post-treatment
    g. 5 hours post-treatment
2. Is there a significant difference in degree of inflammation of subjects after induction of inflammation and after 1, 3, 5 and 7 days post-treatment?
3. Is there a significant difference in the degree of inflammation of the different treatment groups 1, 3, 5 and 7 days post-treatment?
    a. 300mg/kg *P. amarus* extract

b. 150mg/kg *P. amarus* extract
c. 100mg/kg *P. amarus* extract
d. 75mg/kg *P. amarus* extract
e. Positive control (25mg/kg Diclofenac Sodium)
f. Negative control

Hypotheses

1. There is no significant difference in the degree of inflammation of subjects after induction of inflammation and after 1, 3, 5 and 7 days post-treatment.
2. There is no significant difference in the degree of inflammation of the different treatment groups 1, 3, 5 and 7 days post-treatment.

.

Significance of the Study

This research study helps to identify the medicinal plant Sampa-sampalukan (*Phyllantus amarus*) used for the treatment of inflammation. The study will be beneficial to the community through the increasing awareness on the available herbal alternative management in the treatment of inflammation. This study will be conducted to see the importance of herbal plants being seen in the street which can benefit the community. Thus, the study shall be providing an accessible and safer therapy for people suffering from inflammation and ulcer especially to those who are in remote areas. The study will be beneficial for the community especially those in long-term treatment already by providing natural remedy but have lesser side effects than the standard drug and affordable treatment for inflammation. This study is also beneficial in the progress of Pharmacy knowledge and development in the Philippines through the use of herbal plants alternative source of medicine. This research study is important to further help other researchers as guide line or reference to conduct their new researches or in validating of other research studies effectively.

Literature Review

Sampa-sampalukan (*Phyllantus amarus*)

Phyllantus amarus is a member of the Euphorbiaceae family (Spurge family), which groups over 6500 species in 300 genera. Euphorbiaceae is a large family of upright or prostrate herbs or shrubs, often with milky acrid juice (Lewis, 1977) and is mainly a pan-tropical family with some species either more or less temperate. Numerous species of this family are native to North, Central and South America (Unander, 1995). The plants are monoecious or homogamous; leaves are simple, alternate or opposite, some are leathery; flowers are very small and diclinous, they cluster in cup-shaped structures, greenish, often with glands. The fruit is a three-lobed capsule extending from the cup and commonly the long stalk pendant (Boer, 1976; Lewis, 1977). The name ‘Phyllanthus’ means “leaf and flower”

because the flower, as well as the fruit, seems to become one with the leaf (Cabieses, 1993). *Phyllanthus amarus* is widely distributed in all tropical regions of the planet. Paleobotanical studies have not found the exact geographic origin of this plant. This plant may be indigenous to the tropical Americas (Cabieses, 1993; Morton, 1981; Tirimana, 1987), the Philippines or India (Cabieses, 1993, Chevallier, 2000). *Phyllantus amarus* can be found in all the tropical regions of the world: through the roads, valleys, on the riverbanks and near lakes. Phyllanthus amarus reaches a length of 60 cm, the fruits are larger, and the seeds are dark brown and warty (Morton, 1981)

Medical Benefits of *Phyllantus amarus*

All plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Traditional medicine practitioners, in mainly, developing countries have used herbal medicines to treat various ailments including pain and inflammation (Martini-Bettolo, 1980). One of it is *Phyllantus amarus* a world renowned botanical which has been used since ages because of its rich medicinal value and ethnomedical importance. (Ankur et al., 2011) It is widely distributed as a weed in cultivated and waste land. *Phyllantus amarus* is non-toxic and so, its use in ethno medicine is relatively safe (Ajaiyeoba& Kingston, 2006). *Phyllantus amarus* leaves is useful in gastropathy, diarrhea, dysentery, intermittent fevers, opthalmopathy, scabies, ulcers and wounds It has been noted hepatoprotective, anti-inflammatory, analgesic, antipyretic, antiviral and anti microbials (Mehta et al., 2016)

Anti-inflammatory Activity

Studies showed that the soft drink leaf extract of *Phyllantus amarus* exhibits anti-inflammatory and analgesic potentials. The anti-inflammatory and analgesic effects are even higher than those of ibuprofen, the standard anti-inflammatory drug used (Adedapo & Fuegbe, 2013). *Phyllantus amarus* in a form of aqueous extract has been reported to show an anti- inflammatory effect. The extract has been found to sufficiently inhibit the action of inflammatory cells including bradykinins, prostaglandins & serotonin.

Traditional and Alternative Use

Phyllanthus amarus herb has found its traditional usefulness in several health problems such as diarrhoea, dysentery, dropsy, jaundice, intermittent fevers, urinogenital disorders, scabies and wounds. Further, these are used in the treatment of kidney problems, urinary bladder disturbances, pain, gonorrhea, diabetes and chronic dysentery. Topically, it is used for several skin problems ranging from skin ulcers, sores, swelling and itchiness, wounds, bruises, scabies, ulcers and sores, edematous swellings, tubercular ulcers, ringworm, scabby and crusty lesions. Its effect in excretory system is due to its antiurolithic property and is

used in the treatment of kidney/gallstones, other kidney related problems, appendix inflammation and prostate problems.

Traditional and Alternative Medicine Act of 1997

Using herbal medicine was a practice long time ago. The community utilizes their available resources in order to prevent and manage their diseases such as infections. Herbal medications are not only considered for their effective and cost-effective way of preventing or managing infections, but also, due to their constant availability in the community.

Republic Act 8423 (RA 8423) also known as the Traditional and Alternative Medicine Act of 1997, focuses on developing different traditional health-related management in the country.Drugs for prevention, cure, lessening signs and symptoms, diagnosis and maintaining a healthy lifestyle with lower price are needed to be explored and developed. The alternative medications undergo methods of proper compounding. This law encourages the indigenous people to share their traditional medicines and for people to study more about the safety and effectiveness of these alternative medicines. The health care professionals should become aware of these alternative medications and promote to their patients. By this, our countrymen would encounter more alternative medicines coming from that cost much lesser than the existing drugs. The cheaper the medicines get, the more patients will comply with medication (Nolledo, 2015).

Research Paradigm

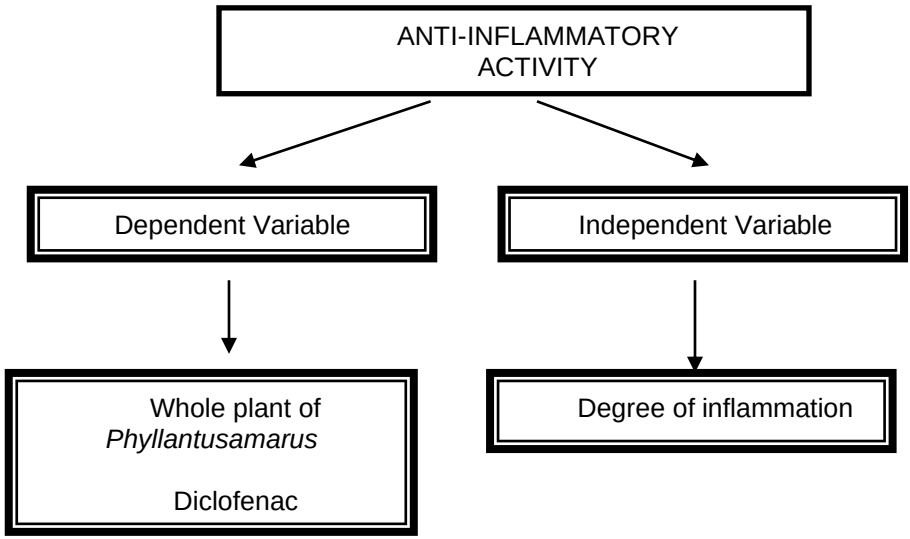

***Figure 1**. Simulacrum of the Study*

The above structure shows that *Phyllantus amarus* (whole plant) and Diclofenac would be used as the experimental control and positive control, respectively.

METHODS

Locale of the Study

The study was the conducted in Philippine Institute of Traditional and Alternative Health Care (PITAHC) - Cagayan Valley Herbal Processing Plant.

Sampling Technique

Taxonomic Identification and Authentication was made at the Bureau of Plant Industry (BPI) – Department of Agriculture, Malate, Manila. Adequate quantities of whole *Phyllanthus amarus* plant were collected in the month of February 2017 from Penablanca, Cagayan.

Drugs and Chemicals

Diclofenac was obtained from a drugstore. Carrageenan was obtained from the Simulation Laboratory of University of Saint Louis Tuguegarao. All drugs and chemicals used for the extraction and assay of extracts found in Sampa-sampalukan (*Phyllanthus amarus)* were of analytical grade unless stated for other specifications needed for the experimentation.

Subjects of the Study

Male wistar rats were used as an experimental animal model. The rats weighted 200±15 grams prior to the initiation of the experiment. The male Wistar rats were procured in Philippine Institute of Traditional and Alternative Health Care (PITAHC) - Cagayan Valley Herbal Processing Plant) located in Carig Regional Center, Tuguegarao City, Cagayan, Philippines for one (1) month.

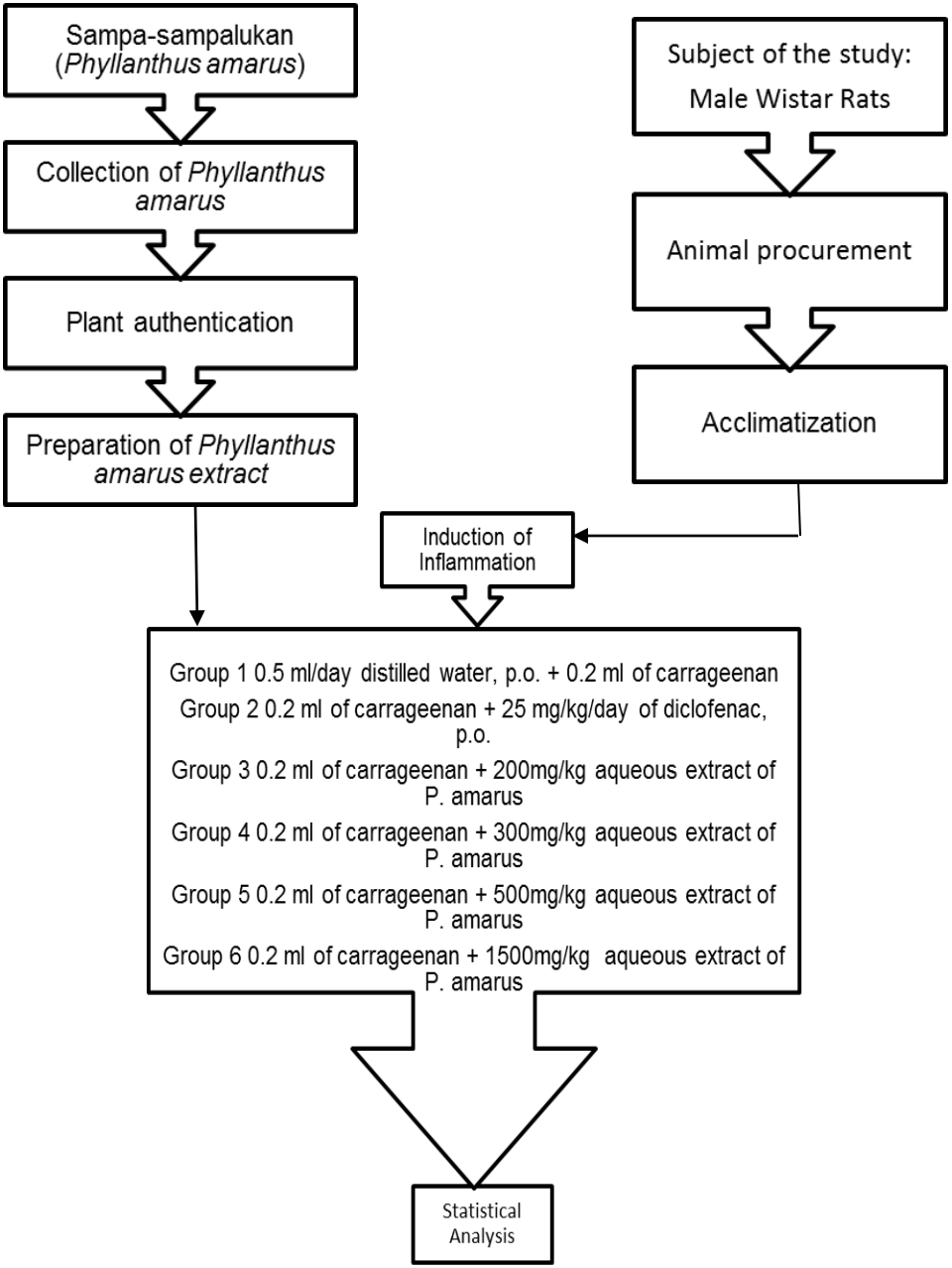

Figure 2. *Methodological Flowchart*

Data Gathering

1. Collection of Plant Material

The whole plant samples were collected at Penablanca, Cagayan in March 2018.The authentication of the plant model was done by Bureau of Plant Industry (BPI) – Department of Agriculture, Malate, Manila.

The collected plants were initially cleaned immediately with tap water and with distilled water thereafter. The whole plant was shade-dried for a week and further crushed into powder; stored in a tight container with label and then stored at controlled room temperature until extraction.

2. Preparation of Aqueous Extracts

The whole plant was dried under shade and grounded into powder. The powdered plant material (200 g) was dissolved in 1 liter of distilled water and filtered twice to remove any residues.

3. Phytochemical Analysis

Sampa-sampalukan whole plant extract was screened of its phytochemical constituent to confirm the presence of alkaloids. Phytochemical test was conducted at Pharmacy Laboratory of University of Saint Louis Tuguegarao.

3.1. Detection of alkaloids

Mayer's Test .One ml of the aqueous extract was added to 2 mL of concentrated hydrochloric acid. Then few drops of Mayer's reagent were added. Presence of brown precipitate indicates the presence of alkaloids.

4. Bioassay

4.1. Acclimatization

A total of 24 rats with a weight range of 100-150 grams purchased from an authorized breeder of rats used in the experimentation were kept in cages. All animals were fed with commercially formulated rat feeds and given with water. The rats were acclimatized for a period of 4 weeks prior to the initiation of experiment.

4.2. Induction of Paw Edema

Male wistar rats weighing 200±15 grams were grouped in six (6) of four (4) animals per group for each dose according to the carrageenan-induced paw

edema. The carrageenan solution (0.1 ml of 1% w/v solution in distilled water) was injected in the sub planted region of right paw of each rat.

4.3. Measurement of Paw-Edema

The swelling of the paws was measured by volume displacement in one hour interval.

Ethical Consideration

Prior to the experimentation phase, the researcher requested permission from the University Research Ethics Board for the ethical clearance and it was approved.

The authentication of the plant model was done by Bureau of Plant Industry (BPI) – Department of Agriculture, Malate, Manila.

The researcher also requested permission from PITAHC regarding the housing of the animal models, in which proper cages and disposal of animal models was taken into consideration to avoid contamination and spread of diseases.

The experiment was carried out according to the Regulatory Services of Bureau of Animal Industry-Department of Agriculture approving all the procedures and having the permission to kill the said animal for the conduction of experiment.

Disposal of the Experimental Animals

The experimental animals were surrendered in the Philippine Institute of Traditional and Alternative Heath Care (PITAHC) - Cagayan Valley Herbal Processing Plant for proper disposal corresponding to their policy.

Data Analysis

Data of paw thickness were analyzed using One-way Analysis of Variance (ANOVA) followed by using LSD as post hoc test. P-value> 0.05 was considered significant.

RESULTS

Table 1. *Degree of Paw Edema/ Inflammation (Volume of Water Displacement) of the Different Treatment Groups Pre and Post-Treatment*

Treatment Groups	After Carrageenan Administration (mean)	1 Hour (mean)	2 Hours (mean)	3 Hours (mean)	4 Hours (mean)	5 Hours
Experimental group 1 (200mg/kg)	1.8000	1.5000	1.1000	.9000	.7000	.4750
Experimental group 2 (300mg/kg)	1.9000	1.4500	1.1500	.9750	.6500	.5000
Experimental group 3 (500mg/kg)	1.9500	1.5000	1.1500	.9250	.6000	.3750
Experimental group 4 (1500mg/kg)	1.9000	1.6500	1.2500	.9000	.7250	.5000
Positive (Diclofenac 25mg/kg)	1.7250	1.4000	1.1250	.7500	.5500	.4500
Negative control (plain water)	1.7500	1.9250	1.9500	2.0250	2.1000	2.1500

The table above presents the measurement of paw edema which was used as measure for assessing degree of inflammation manifested by the subjects. It can be further observed that a decreasing trend in the degree of inflammation can be observed in all treatments except in the negative control where there is an increase in paw edema after administration of Carrageenan.

Table 2.1. *Test of Significant Difference of the Degree of Inflammation of Subjects under the Experimental Group 1 after Induction of Inflammation and Post-treatment*

Pairs	t-value	p-value	Decision
Post-Carrageenan Administration- 1 hour Post Treatment	2.828	.066	Accept Ho
Post-Carrageenan Administration- 2 hours Post Treatment	5.422	.012	Reject Ho
Post-Carrageenan Administration- 3 hours Post-Treatment	9.000	.003	Reject Ho

Post-Carrageenan Administration-4 hours Post Treatment	19.053	.000	Reject Ho
Post-Carrageenan Administration-5 hours Post Treatment	17.667	.000	Reject Ho

The table shows that the degree of paw edema or inflammation of the test subjects decreased significantly after 2, 3, 4, and 5 hours of administering 200mg/kg dosage of the *P. amarus* extract.

Table 2.2. *Test of Significant Difference of the Degree of Inflammation of Subjects under the Experimental Group 2 after Induction of Inflammation and Post-treatment*

Pairs	t-value	p-value	Decision
Post-Carrageenan Administration- 1 hour Post Treatment	2.377	.098	Accept Ho
Post-Carrageenan Administration- 2 hours Post Treatment	4.392	.022	Reject Ho
Post-Carrageenan Administration- 3 hours Post-Treatment	8.343	.004	Reject Ho
Post-Carrageenan Administration-4 hours Post Treatment	9.934	.002	Reject Ho
Post-Carrageenan Administration-5 hours Post Treatment	17.146	.000	Reject Ho

The table shows that the degree of paw edema or inflammation of the test subjects decreased significantly after 2, 3, 4, and 5 hours of administering 300mg/kg dosage of the *P. amarus* extract.

Table 2.3. *Test of Significant Difference of the Degree of Inflammation of Subjects under the Experimental Group 3 after Induction of Inflammation and Post-treatment*

Pairs	t-value	p-value	Decision
Post-Carrageenan Administration- 1 hour Post Treatment	3.576	.037	Reject Ho
Post-Carrageenan Administration- 2 hours Post Treatment	5.657	.011	Reject Ho
Post-Carrageenan	5.308	.013	Reject Ho

Administration- 3 hours Post-Treatment			
Post-Carrageenan Administration-4 hours Post Treatment	6.088	.009	Reject Ho
Post-Carrageenan Administration-5 hours Post Treatment	6.678	.007	Reject Ho

The table shows that the degree of paw edema or inflammation of the test subjects decreased significantly after 1, 2, 3, 4, and 5 hours of administering 500mg/kg dosage of the *P. amarus* extract.

Table 2.4. *Test of Significant Difference of the Degree of Inflammation of Subjects under the Experimental Group 4 after Induction of Inflammation and Post-treatment*

Pairs	t-value	p-value	Decision
Post-Carrageenan Administration- 1 hour Post Treatment	5.000	.015	Reject Ho
Post-Carrageenan Administration- 2 hours Post Treatment	13.000	.001	Reject Ho
Post-Carrageenan Administration- 3 hours Post-Treatment	12.247	.001	Reject Ho
Post-Carrageenan Administration-4 hours Post Treatment	9.945	.002	Reject Ho
Post-Carrageenan Administration-5 hours Post Treatment	11.431	.001	Reject Ho

The table shows that the degree of paw edema or inflammation of the test subjects decreased significantly after 1, 2, 3, 4, and 5 hours of administering 1500mg/kg dosage of the *P. amarus* extract.

Table 2.5. *Test of Significant Difference of the Degree of Inflammation of Subjects under the Positive Control Treatment Group after Induction of Inflammation and Post-treatment*

Pairs	t-value	p-value	Decision
Post-Carrageenan Administration- 1 hour Post Treatment	9.000	.003	Reject Ho
Post-Carrageenan Administration- 2 hours Post	4.899	.016	Reject Ho

Treatment			
Post-Carrageenan Administration- 3 hours Post-Treatment	8.253	.004	Reject Ho
Post-Carrageenan Administration-4 hours Post Treatment	8.182	.004	Reject Ho
Post-Carrageenan Administration-5 hours Post Treatment	8.540	.003	Reject Ho

It can be gleaned on the table above that the degree of paw edema or inflammation of the test subjects significantly decreased after 1, 2, 3, 4 and 5 hours of administering the combined *2.5mg/kg of Diclofenac Sodium*.

Table 2.6. *Test of Significant Difference of the Degree of Inflammation of Subjects under the Negative Control Treatment Group after Induction of Inflammation and Post-treatment*

Pairs	t-value	p-value	Decision
Post-Carrageenan Administration- 1 hour Post Treatment	-2.049	.133	Accept Ho
Post-Carrageenan Administration- 2 hours Post Treatment	-2.828	.066	Accept Ho
Post-Carrageenan Administration- 3 hours Post-Treatment	-3.667	.035	Reject Ho
Post-Carrageenan Administration-4 hours Post Treatment	-5.422	.012	Reject Ho
Post-Carrageenan Administration-5 hours Post Treatment	-4.382	.022	Reject Ho

It can be gleaned on the table above that the degree of paw edema or inflammation of the test subjects did not significantly change after 1 and 2 hours of administering sterile water. Moreover, the degree of paw inflammation/ edema significantly increased after 3, 4 and 5 hours of administering sterile water.

Table 3. *Test of Significant Difference in the Degree of Inflammation of the Different Treatment Groups 1, 3, 5 and 7 days Post-treatment*

Pairs	F-value	p-value	Decision
1 hour Post-treatment	1.431	.261	Accept Ho

2 hours Post-treatment	5.521	.003	Reject Ho
3 hours Post-treatment	26.777	.000	Reject Ho
4 hours Post-treatment	45.285	.000	Reject Ho
5 hours Post-treatment	46.841	.000	Reject Ho

The table above shows that there is a significant difference in the degree of paw edema/ inflammation among the different treatment groups (negative control, positive control, experimental groups 1, 2, 3 and 4) after administration of the respective treatments for 1, 2, 3, 4 and 5 hours.

Table 4.1. *Post-Hoc Analysis of the Test of Significant Difference in the Degree of Inflammation of the Different Treatment Groups 1 hour Post-treatment*

	Mean	**Exp. Group 1**	**Exp. Group 2**	**Exp. Group 3**	**Exp. Group 4**	**Positive Control**	**Negative control**
Exp. Group 1	1.5000						
Exp. Group 2	1.4500	.829					
Exp. Group 3	1.5000	.666	.829				
Exp. Group 4	1.6500	.287	.391	.518			
Positive Control	1.4000	.666	.829	1.000	.518		
Negative control	1.9250	.078	.052	.078	.243	.033*	

**The mean difference is significant at the 0.05 level*

The table above shows that after 1 hour of treatment, the 200mg/kg *P. amarus* extract manifested significantly the same anti-inflammatory effect as the positive control (Diclofenac Sodium).

Table 4.2. *Post-Hoc Analysis of the Test of Significant Difference in the Degree of Inflammation of the Different Treatment Groups 2 hours Post-treatment*

	Mean	**Exp. Group 1**	**Exp. Group 2**	**Exp. Group 3**	**Exp. Group 4**	**Positive Control**	**Negative control**
Exp. Group 1	1.1000						
Exp. Group 2	1.1500	.803					
Exp. Group 3	1.1500	.803	1.000				

Exp. Group 4	1.2500	.458	.619	.619			
Positive Control	1.1250	.901	.901	.901	.535		
Negative control	1.9500	.000*	.001*	.001*	.002*	.001*	

**The mean difference is significant at the 0.05 level*

The table above shows that after 2 hours of treatment, all dosage concentrations of *P. amarus* extract manifested significantly the same anti-inflammatory effect as the positive control (Diclofenac Sodium).

Table 4.3. *Post-Hoc Analysis of the Test of Significant Difference in the Degree of Inflammation of the Different Treatment Groups 3 hours Post-treatment*

	Mean	**Exp. Group 1**	**Exp. Group 2**	**Exp. Group 3**	**Exp. Group 4**	**Positive Control**	**Negative control**
Exp. Group 1	.9000						
Exp. Group 2	.9750	.566					
Exp. Group 3	.9250	.848	.701				
Exp. Group 4	.9000	1.000	.566	.848			
Positive Control	.7500	.258	.096	.189	.258		
Negative control	2.0250	.000*	.000*	.000*	.000*	.000*	

**The mean difference is significant at the 0.05 level*

The table above shows that after 2 hours of treatment, all dosage concentrations of *P. amarus* extract manifested significantly the same anti-inflammatory effect as the positive control (Diclofenac Sodium).

Table 4.4. *Post-Hoc Analysis of the Test of Significant Difference in the Degree of Inflammation of the Different Treatment Groups 4 hours Post-treatment*

	Mean	**Exp. Group 1**	**Exp. Group 2**	**Exp. Group 3**	**Exp. Group 4**	**Positive Control**	**Negative control**
Exp. Group 1	.7000						
Exp. Group 2	.6500	.695					

Exp. Group 3	.6000	.436	.695				
Exp. Group 4	.7250	.844	.558	.333			
Positive Control	.5500	.248	.436	.695	.180		
Negative control	2.1000	.000*	.000*	.000*	.000*	.000*	

**The mean difference is significant at the 0.05 level*

The table above shows that after 2 hours of treatment, all dosage concentrations of *P. amarus* extract manifested significantly the same anti-inflammatory effect as the positive control (Diclofenac Sodium).

Table 4.5. *Post-Hoc Analysis of the Test of Significant Difference in the Degree of Inflammation of the Different Treatment Groups 5 hours Post-treatment*

	Mean	**Exp. Group 1**	**Exp. Group 2**	**Exp. Group 3**	**Exp. Group 4**	**Positive Control**	**Negative control**
Exp. Group 1	.4750						
Exp. Group 2	.5000	.863					
Exp. Group 3	.3750	.493	.393				
Exp. Group 4	.5000	.863	1.000	.393			
Positive Control	.4500	.863	.730	.606	.730		
Negative control	2.1500	.000*	.000*	.000*	.000*	.000*	

**The mean difference is significant at the 0.05 level*

The table above shows that after 2 hours of treatment, all dosage concentrations of *P. amarus* extract manifested significantly the same anti-inflammatory effect as the positive control (Diclofenac Sodium).

DISCUSSION

Phyllantus amarus in a form of aqueous extract has been reported to show an anti- inflammatory effect. The extract has been found to sufficiently inhibit the action of inflammatory cells including bradykinins, prostaglandins & serotonin (Adedapo & Ofuegbe, 2013). The major chemical constituents present in Phyllanthus amarus are mainly alkaloids, in the form of lignins, which is phyllanthin and hypophyllanthin according to Adedapo and Ofuegbe (2013) and Husain (2014).

The two main lignins present in Phyllanthus amarus are the ones responsible for the anti-inflammatory activity. Phytochemical screening of alkaloids rendered a brown precipitate which indicates a positive result in the whole plant aqueous extract of Phyllanthus amarus.

Diclofenac is used as a reference standard in identifying the anti-inflammatory activity of *Phyllantus amarus* aqueous extract. Distilled water also served as the negative control. The agent used for testing these anti-inflammatory agents is Carrageenan which is known for not having systemic effects.

In the experimentation phase, inflammation in the right paw edema of the Wistar rats was measured by volume displacement. It showed that there is gradual decrease in inflammation in the treatment groups within 6 hours proving that the *Phyllantus amarus* have anti-inflammatory activity. In the carrageenan induced paw edema, the animals that were treated with Phyllanthus amarus aqueous extract has no significant difference at 200mg/kg, 300mg/kg, 500mg/kg, and 1500 mg/kg when compared with the reference standard which is the Diclofenac 25mg/kg (Table 3B). However, the ideal dose of *Phyllanthus amarus* based on the ($p<.05$) mean difference of -0.500 the groups was the Phyllanthus amarus 300 mg/kg.

CONCLUSION

The present study showed that the aqueous extract of *Phyllantus amarus* exhibits a comparable result in terms of the anti-inflammatory activity with the reference standard Diclofenac. It has an inhibitory effect on carrageenan induced paw edema after 6 hours with the dose of 200mg/kg, 300mg/kg, 500mg/kg, and 1500mg/kg respectively. It also showed that there is no significant difference in the anti-inflammatory activity between the positive control, diclofenac and the experimental control, whole plant extract of *Phyllantus amarus* in male wistar rats.

RECOMMENDATION

This research study recommends the following:

a. Conduct phytochemical screening of the plant
b. Conduct histopathological study to see if there is a gastroprotective property of the plant;
c. Use other Non Steriodal Anti-inflammatory Drug as reference standard and other solvents.

REFERENCES

Adedapo, A. A., & Ofuegbe, S. O. (2013). Anti-inflammatory and Analgesic activities of Soft Drink Leaf Extract of Phyllantus amarus in Some Laboratory Animals. *British Biotechnology Journa*l, 191-204.

Amabeoku, G. J. &Kabatende, J., (2012). Anti nociceptive and Anti-inflammatory Activities of Leaf Methanol Extract of Cotyledon orbiculata L. (Crassulaceae). *Advances in Pharmacological Sciences*, 1-6

Andaya, A., Herrera, M.J., Pajarillo, K.C., &Wanya, F.M. (2017). Anti-inflammatory Activity of Madre de cacao (Gliricidia sepium) Leaf Extract. 1-49

Brunton, L., Parker, K., Blumenthal, D., & Buxton, I. (2008).Goodman & Gilman's Manual of Pharmacology and Therapeutics. USA: The McGraw-Hill Companies, Inc.

Diyva, K., Ankkur, R., Seema, R., & M.U., K. (2011).Phyllantusamarus: An ample therapeutic potential herb. I*nternational Journal of Research in Ayurveda & Pharmacy*, 2(4), 1096-1099.

Furst, D. E., Ulrich, R. W., & Prakash, S. (2001). Nonsteroidal anti-inflammatory drugs, disease-modifying antirheumatic drugs, nonopioid analgesics, and drugs used in gout. *Basic and clinical pharmacology*, *8*, 596-624.

Joseph, B., & Raj, S. (2010). Phytopharmacological properties of Ficcusracemosa Linn: An Overview. Int. J. Pharma. Sci. Rev. Res., 3, 134-138.

Oseni, L.A., Amiteye, D.,Antwi, S., Tandoh, M., &Aryitey, G.M. (2013). Preliminary in vivo evaluation of anti-inflammatory activities of aqueos and ethanolic whole plant extracts of Phyllanthus fraternus on Carageenan-induced paw Oedema in Sprague-Dawley Rats*. Journal of Applied Pharmceutical Science,* 3, 62-65.

Mahat, M. A., & Patil, B. (2007). Evaluation of Anti-infammatory activity of ethanol Extract of Phyllanthus amarus in Experimental animal models. *Indian Journal of Pharmaceutical Sciences*, 33-36.

Mohammed, A. (2008). Pharmacognosy, and Phytochemistry Edition 1, Volume 1. Delhi: cbs publishers and distributors.

Mostofa, R., Ahmed, S., Begum, M., Rahman, S., Begum, T., Ahmed, S. U., Tuhin, R., Das, M., Hossain, A., Sharma, M., & Begum, R. (2017). Evaluation of anti-inflammatory and Gastroprotective activity of Phyllanthusniruri L. (Euphorbiaceae) leaves in Experimental rats. *BMC complementary and Alternative Medicine*, 1-10.

Sarin, B., Verma, N., Martín, J. P., & Mohanty, A. (2014). An overview of important ethnomedicinal herbs of Phyllanthus species: present status and future prospects. *The Scientific World Journal*, *2014*.

Sen, A., & Batra, A. (2013). The study of in vitro and in vivo antioxidant activity and total phenolic content of Phyllanthus amarus Schum. & Thonn.: A medicinally important plant. *International Journal of Pharmacy and Pharmaceutical Sciences*, *5*(3), 942-947.

Shokunbi, O. S. & Odetola, A. A. (2008). Gastroprotective and antioxidant activities of Phyllanthus amarus extracts on absolute ethanol-induced ulcer in albino rats. *Journal of Medicinal Plants Research*, 2(10), 261-267

Sirajudeen, K. N. S., Sulaiman, S. A., Madhavan, M., Ismail, Z., Swamy, M., Ismail, M. L., & Yaacob, M. (2006). Safety evaluation of aqueous extract of leaves of a plant Phyllanthus amarus, in rat liver. *African Journal of Traditional, Complementary and Alternative Medicines*, *3*(4), 78-93.

Verma, S., Sharma, H., & Garg, M. (2014).Phyllantus amarus: A review. *Journal of Pharmacognosy and Phytochemistry*, 18-22.

Whalen, P. B. (2015). Anti-inflammatory, Antipyretic, and Analgesic Agents. In P. B. Karen Whalen, Lippincott Illustrated Revies: Pharmacology Sixth Edition (p. 447). China: Wolters Kluwer.

Webster, G. L. (1957). A monographic study of the West Indian species of Phyllantus.Journal of the Arnold Arboretum, vol. 38, 295-373.