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Original Research Paper

Pharma

POTENTIAL ANTI-INFLAMMATORY EFFECT OF ETHANOLIC LEAF EXTRACT OF ASPLENIUM NIDUS ON CARRAGEENAN-INDUCED PAW EDEMA OF SWISS MICE.

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ABSTRACT *Asplenium nidus* (Palpak-Lauin) is an epiphytic medicinal plant used traditionally in tropical parts of Southeast Asia for curing skin diseases, generalized weakness, halitosis, as asthma, labor pain, elephantiasis, mouth sores and inflammatory skin diseases and jaundice. This study determined the presence of Quercetin flavonoid and evaluated the anti-inflammatory effects of the ethanolic leaf extract of *Asplenium nidus*. Fourier Transform Infrared Spectroscopy was used to determine the presence of Quercitrin in the extract which itself is a precursor for Quercetin. The anti-inflammatory activity of the compound was evaluated by using the carrageenan induced mice paw edema. Carrageenan is a polysaccharide of sulfated galactose units which is derived from Irish Sea Moss, which releases histamine and serotonin in the first hour, followed by prostaglandins, protease and lysosome in the second hour. The doses of the extract were determined by Approximate Effective Dose (AED) and Effective Dose 50 (ED50) method. Our results suggest that the ethanoic leaf extract of *Asplenium nidus* possess anti-inflammatory properties which may justify its traditional use in several communities.

KEYWORDS : Fourier Transform Infrared Spectroscopy, Approximate Effective Dose, Effective Dose 50, Quercitrin, Flavanoid, Asplenium nidus

INTRODUCTION-

Inflammation is a broad term referring to complex changing responses which are beneficial in wound healing and disease control but can be pathological in many chronic disease states by inflicting excessive collateral damage. Studies have shown that inflammation can serve as independent risk factor for mortality in total healthy adult population. [1] that is why drugs are used to control and reduce inflammation. Currently, the pharmacological management used to reduce inflammation are steroidal drugs, NSAIDS and immuno modulators.

These drugs comes with gastrointestinal and the cardiovascular risks and adverse effects. [2] Thus, we need to consider natural anti-inflammatory therapy to achieve response and the lower the degree of unwanted side effects. Studies on *Asplenium nidus* have shown anti-bacterial, anti-oxidant and anti-cancer phytochemical. The anti-oxidant and anti-inflammatory properties are mainly due the most extracted flavonoid gliricidin-7-O-hexoside and quercetin-7-O-rutinoside. Most *in vitro* researches done previously have shown that Quercetin occupy anti-inflammation and immunological improvement.[3] *Asplenium nidus* is consumed as food in various parts of Southeast Asia.[4]This study aims to see potential anti-inflammatory effects of ethanolic leaf extract of *Asplenium nidus* on reducing the inflammation on carrageenan induced paw edema of Swiss mice.

Asplenium Nidus-

METHODOLOGY-

Plant Collection and Authentication

Asplenium nidus was identified and authenticated by the botanist Dr. Abad from Davao Doctors College.

Preparation of the Plant Extract

About 2kg of plant leaves were dried in shade for 14 days then grinded which soaked in 80 % ethanol for 3 days. Filtration was done by cheese cloth and the filtrates were concentrated in a rotary evaporator at a 45C. The ethanolic leaf extract was refrigerated and stored properly to prevent the growth of the microorganisms. The concentration of the stock solution was 30 grams for 45 ml. Therefore the concentration was 667 mg/ml.

Drugs and Chemicals

Carrageenan, Diclofenac Sodium and Normal Saline was purchased from Chemvest, Davao City.

Flavonoid assay

Flavonoids were determined using the colorimetric assay. Diluted ammonia (5 ml) solution was mixed with the plant extract followed by concentrated H2SO4. A yellow coloration of the extract indicated the presence of the flavonoids.

Quantitative analysis of the flavonoids

The total flavonoid content in *Asplenium nidus* was determined using aluminum chloride (AlCl3) colorimetry assay. The quantity of the flavonoid Quercetin was measured by High-Performance Liquid Chromatography (HPLC) which was a form of column chromatography.

Fourier Transform Infrared Spectroscopy

FTIR is used to identify chemicals by using an infrared light source based on their wavelengths. There is a peak of a component in the 5th minute at 255nm. Since Quericitin is derived from the compound Querictrin, we can conclude that Quercitrin has Anti-inflammatory effect.

SCORE	NAME
851	L-Arabinitol

847	D-(+)-Raffinose
835	Quercitrin
834	DL-Arabinitol

Determination of peak effect of carrageenan on mice

Since Carrageenan is known to produce inflammation, the time at which it shows maximum inflammation (maximum paw edema) should be determined. In 100 ml of distilled water, 1 gm of carrageenan was dissolved to make 1% carrageenan suspension.

The carrageenan suspension was injected subcutaneously into the plantar surface of three mice to induce the edema. The paw thickness was measured for every half an hour until paw edema subsided. The maximum paw edema was observed at 2nd hour after carrageenan induction. Therefore to reduce the inflammation, animals can be treated with Diclofenac and plant extract of *Asplenium nidus* orally as soon as carrageenan is injected.

Determination of Approximate Effective Dose (AED)

Determination of Approximate effective dose included 6 groups with 2 mice in each group. The concentrations were the following:

- 3mg/kg
- 4.45mg/kg
- 17.71mg/kg
- 70.48mg/kg
- 280 mg/kg and
- 1114.4mg/kg

The percentage of inhibition of the edema was measured by the following formula

% inhibition = 100 (1-(α-x/b-y))

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Where, α = mean paw volume of treated animals after 2nd hour of carrageenan injection; x = mean paw volume of treated animals before carrageenan injection; b = mean paw volume of control animals after 2nd hour of carrageenan injection; y = mean paw volume of control animals before carrageenan injection

The concentration at which there was a 33% reduction in the paw edema was considered as clinically significant. The upper limit was 4.45mg/kg and the lower limit was 3mg/kg. The AED factor was found to be 0.29.

Determination of Effective Dose (Ed50)

Using the AED factor the following doses were calculated from the lower limit to find the Ed50.

GROUP A - 3.29mg/kg
GROUP B - 3.58mg/kg
GROUP C - 3.87mg/kg
GROUP D - 4.16mg/kg
GROUP E – 4.45mg/kg

Five groups of 4 mice each were treated with the above mentioned doses. Significant reduction of paw edema was seen in GROUP A (3.29mg/kg) and GROUP D (4.16 mg/kg). Among both doses 3.29mg/kg was considered as the effective and baseline dose.

AED factor=(upper limit - lower limit) ÷5 (arbitrary number).Experimental group

Carrageenan induced paw edema method was performed to evaluate the anti-inflammatory effect of the leaf extract. Twenty five Swiss mice were divided into five groups; each group was comprised of five mice. The groups were designated as to the following:

Group 1 - treated with saline solution (0.1ml)
Group 2 - treated with Diclofenac sodium (10 mg/kg po)
Group 3 - 3.29 mg/kg, low dose of the A.nidus extract
Group 4- 6.58 mg/kg, medium dose of the A.nidus extract
Group 5- 9.87 mg/kg, high dose of the A.nidus extract

1% Carrageenan suspension was injected subcutaneously into the plantar surface to induce edema. The paw thickness was measured by the Vernier caliper every half an hour till the paw edema subsided for about 6 hours.

Single blind study was used in the experiment.

RESULTS-

Table – 1 Mean Paw Thickness at 1st and 2nd after the Administration of carrageenan in Different Treatment Groups

Treatment Group	Dose	Mean ± SD		
		Baseline	2 Hours (END STUDY)	Mean difference
Group 1	Saline solution (0.1 ml)	1.94 ± 0.09	4.20 ± 0.46	2.26
Group 2	Diclofenac (10 mg/kg)	2.20 ± 0.24	3.22 ± 0.40	1.00
Group 3	Asplenium nidus extract Low dose (3.29 mg/kg)	1.76 ± 0.15	2.48 ± 0.26	0.72
Group 4	Asplenium nidus extract Medium dose (6.58 mg/kg)	2.00 ± 0.10	3.20 ± 0.38	1.20
Group 5	Asplenium nidus extract High dose (9.87 mg/kg)	1.98 ± 0.13	3.48 ± 0.55	1.5

The results of **Table 1**- shows that before administration of Carrageenan the average paw thickness of Group 1 is 1.94, Group 2 has an average thickness of 2.20 which is the highest amongst the groups. Group 3 has an average thickness of 1.76 is the lowest amongst the other groups, while Group 4 has 2.00, and Group 5 has 1.98.

2 hrs. after the administration of Carrageenan, the paw thickness increased to 4.2 in Group 1 being the highest increment in paw thickness among other groups, while Group 3 being the lowest measurement in paw thickness which is 2.48. So, the group 3, *Asplenium nidus* extract Low dose (3.29 mg/kg) has the significant reduction in paw edema among the groups.

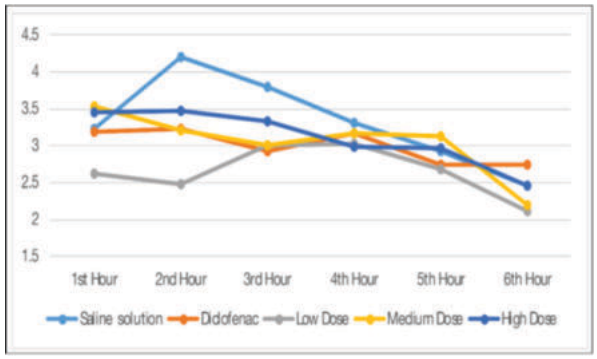

Fig 1 -Mean Paw Thickness from 1st Hour to 6th Hour after the Administration of Carrageenan in Different Treatment Groups

Table-3 Percentage of inhibition of paw thickness among Different treatment groups

Treatment Group	1st hour	2nd hour
Diclofenac	57%	55%
Low Dose	62%	68%
Medium Dose	33%	47%
High Dose	36%	34%

The Tukey HSD multiple comparison results showed there is sufficient evidence to prove that low dose and Diclofenac are significantly different from each at the 2nd hour of administration of carrageenan in experimental groups and there is a significant difference between the treatment groups and the negative control group.

DISCUSSION-

In this study, we reported the anti-inflammatory effect of ethanolic leaf extract of *Asplenium nidus* on Carrageenan induced paw edema of Swiss mice.). In this study, the peak effect of carrageenan was found to be 2 hours after its administration. The Approximate Effective Dose Factor of the ethanolic extract of *Asplenium nidus* was found to be 0.29. The effective and baseline dose was found to be 3.29mg/kg. The analysis of this research was based on the effect of the 3 different doses on paw edema of Swiss mice after 2 hours of administration of carrageenan and compare it with negative and positive controls. The percentage of inhibition of low dose after 2 hours of administration of carrageenan was 68% which was higher than the 55% inhibition of positive control (Diclofenac sodium). FTIR data analysis confirmed the presence of phenolic compound Quercitrin, which is a flavonoid with anti-inflammatory activity that is converted into quercetin which performs anti-inflammatory effect by inhibiting the NF-kB pathway.

CONCLUSION

This study has demonstrated the anti-inflammatory properties of *Asplenium Nidus*. Further research is needed in order to explain and predict how it can work as an alternative medicine for the inflammatory disease. Further research will also make it possible to better understand the mechanisms of action responsible for various pharmacological activities of the plant extract.

Authors Contributions:

Conflict of Interest:

The author declares no conflicts of interest.

Ethical Approvals:

IACUC no 2018-01-004, Research Ethics Committee, Medical School Drive, Bajada, Davao City, Philippines; erc@email.dmsf.edu.ph

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