# IN-VITRO ANTI-INFLAMMATORY ACTIVITY AND QUALITATIVE BIOCHEMICAL ANALYSIS OF NEER PEENISA CHOORANAM- A SIDDHA POLYHERBAL FORMULATION

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***Abstract*- Siddha system of medicine is an integrated part of Indian system of medicine which is rooted in Dravidian culture of the pre-vedic period. Ancient siddha literature numbered the disease as 4448. Neer peenisam (Sinusitis) is one among the disease mentioned in "Yugimuni vaithya kaviyam." Neer peenisa chooranam (NPC) is a Siddha classical polyherbal formulation that has been mentioned in Sarabendra siraroga nidhanam" authored by Vengadarajan S, for the treatment of Neer Peenisam. Aim: The main aim of this study is to analysis the in-vitro anti-inflammatory potential and bio chemical analysis of Neer Peenisa chooranam. Methodology: The present study was undertaken to evaluate anti-inflammatory activity of Neer Peenisa chooranam (NP) by protein (Albumin) denaturation assay. This study also analysed the qualitative analysis of biochemical in polyherbal drug of Neer peenisa choornam. Result: The study result was concluded that Neer Peenisa chooranam has significant Anti- inflammatory activity.**

***Keywords:*** **Neer Peenisam, Sinusitis, Neer Peenisa chooranam, Anti-inflammatory activity.**

## 1) INTRODUCTION

Sinusitis is an inflammation of the mucosal lining of Paranasal sinuses which is characterised by Stuffy or Running nose, Sneezing, cough, Headache, Increased phlegm. It usually occur after the age of 15 years and is diagnosed more frequently in adult women than men. Sinusitis occurs as the result of an inflammatory reaction from a virus, bacteria, or fungus. Acute sinusitis can be triggered by a cold or allergies and chronic sinusitis caused by an infection or growth. The word "Neer peenisam" is correlated with that of Sinusitis.Siddha treatments of sinusitis are very effective for reducing inflammation, runny or blocked nose as well as other symptoms of disease.

Hence this current study was carried out to prove the anti-inflammatory activity of "Neer Peenisa chooranam" by invitro assays and biochemical analysis.

## 2) MATERIALS AND METHODS

### 2.1) Drug selection:

The siddha polyherbal formulation Neer peenisa choornam is mentioned in siddha literature "Sarabendra siraroga nidhanam." (Page no;117)

### 2.2) Ingredients of Neer Peenisa chooranam :

1.Manjal (Curcuma longa L.)
2.Karum jeerakam (Nigella sativa L.)
3.Kazharchi paruppu (Caesalpinea bonducella L.)

### 2.3) Authentication of Raw Drugs:

The ingrediants of polyherbal drugs are authenticated by faculties of Department of Gunapadam, Government siddha medical college and Hospital, Palayamkottai.

### 2.4) Method of Purification:

All the raw drugs are purified as per the methods mentioned in Siddha literature.

### 2.5) Process of drug preparation

The above purified three ingredients were powdered individually and mixed together and stored in air tight container.

### 2.6) Anti-inflammatory activity evaluation using Denaturation Albumin Assay Procedure

In-vitro anti-inflammatory activity of the sample NPC was studied using albumin denaturation technique. The reaction mixture consisting of bovine serum albumin (5% aqueous solution) and the test sample NPC at varying concentration ranges from 100 to

500 µg/ml along with standard Diclofenac sodium at the concentration of 100 µg /ml of final volume. pH was adjusted by using a small amount of 1N Hydrochloric acid. The samples were incubated at 37°C for 20 min and then heated at 57°C for 3 min. After cooling the sample, 2.5 ml of phosphate buffer solution was added into each test tube. Turbidity developed was measured spectrophotometrically at 660 nm, for control distilled water was used instead of test sample while product control tests lacked bovine serum albumin. The experiment was performed in triplicate. The Percentage protection from denaturation is calculated by using the formula.

$$\left[\frac{(A)_{\text{control}} - (A)_{\text{sample}}}{(A)_{\text{control}}}\right] \times 100.$$

2.7) Biochemical Analysis of Neer peenisa Chooranam:
Preparation of the extract:

5 gms of the Neer peenisa choornam was weighted accurately and placed in a 250 ml clean beaker then 50 ml of distilled water is added and dissolved well. Then it is boiled well for about 10 minutes. It is cooled and filtered in a 100 ml volumetric flask and it is made to 100 ml with distilled water. This fluid is taken for analysis.

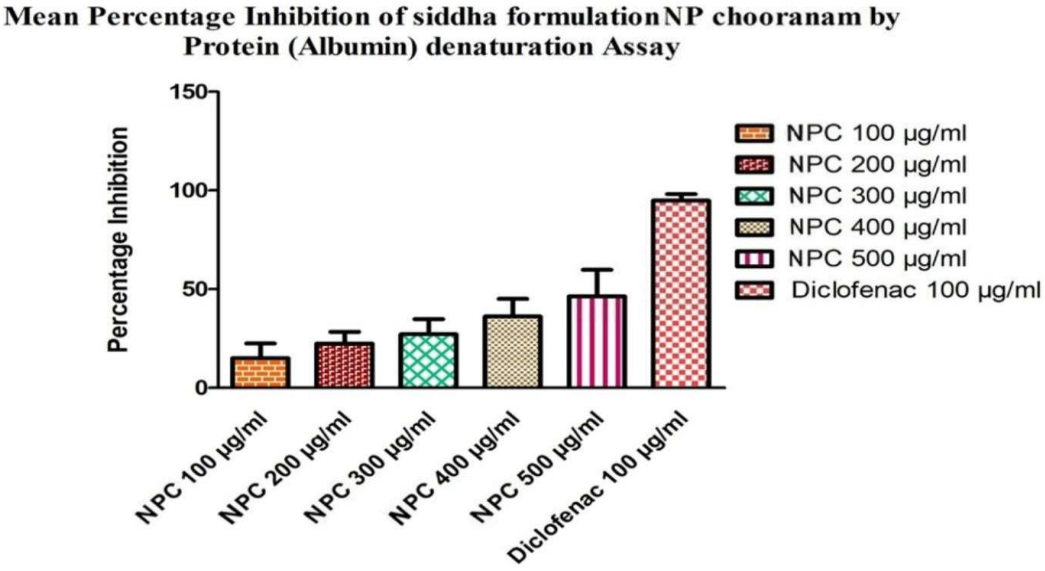
3) **RESULTS**
Statistical analysis of Neer Peenisa Choornam

Results are expressed as Mean ± SD. The difference between experimental groups was compared by One-Way Analysis of Variance (ANOVA) followed by Dunnet Multiple comparison test.

| Concentration in µg/ml | Percentage Inhibition of Protein Denaturation |
|---|---|
| NPC 100 | 15.01 ± 7.517 |
| NPC 200 | 22.48 ± 6.024 |
| NPC 300 | 27.16 ± 7.736 |
| NPC 400 | 36.15 ± 9.061 |
| NPC 500 | 46.49 ± 13.54 |
| Diclofenac sodium (100 µg) | 94.91 ± 3.37 |

Each value represents the mean ± SD. N=3

Percentage Inhibition of Protein Denaturation by NPC and Standard



Biochemical Analysis:

| S. No | Procedure | Observation | Inference |
|---|---|---|---|
| 1. | Test for calcium:<br>2 ml of the above prepared extract taken in a clean test tube. To this add 2 ml of 4% ammonium oxalate solution. | A White precipitate is formed | Presence of calcium |
| 2. | Test for sulphate:<br>2ml of the extract is added to 5% barium chloride solution. | A White precipitate is formed | Presence of sulphate |
| 3. | Test for chloride:<br>The extract is treated with silver nitrate solution. | White precipitate is formed | Presence of Chloride |
| 4. | Test for carbonate:<br>The substance is treated with concentrated HCL. | No brisk effervescence is formed | Absence of carbonate |
| 5. | Test for Starch:<br>The extract is added with weak iodine solution. | Blue colour is formed | Presence of starch |
| 6. | Test for ferric iron:<br>The extract is acidified with glacial acetic acid and potassium ferro cyanide. | No blue colour is formed | Absence of ferric iron |
| 7. | Test for ferrous iron:<br>The extract is treated with concentrated nitric acid ammonium thiocyanate solution. | Blood red colour is formed | Presence of ferrous iron |
| 8. | Test for phosphate:<br>The extract is treated with ammonium molybdate and concentrated nitric acid. | No Yellow precipitate is formed | Absence of phosphate |
| 9. | Test for albumin:<br>The extract is treated with esbach's reagent. | No yellow precipitate is formed | Absence of albumin |
| 10. | Test for tannic acid:<br>The extract is treated with ferric chloride. | No blue black precipitate is formed | Absence of tannic acid |
| 11. | Test for unsaturation:<br>Potassium permanganate solution is added to the extract. | It gets decolourised | Presence of unsaturated compound |
| 12. | Test for the reducing sugar:<br>5 ml of benedict's qualitative solution is taken in a test tube and allowed to boil for 2 minutes and add 8 to 10 drops of the extract and again boil it for 2 minutes. | No Colour changes occurs | Absence of reducing sugar |
| 13. | Test for amino acid:<br>One or two drops of the extract is placed on a filter paper and dried well. After drying, 1% ninhydrin is sprayed over the same and dried it well. | Violet colour is formed | Presence of amino acid |
| 14. | Test for zinc:<br>The extract is treated with Potassium Ferro cyanide. | No white precipitate is formed | Absence of zinc |

## 4) DISCUSSION

Result analysis of NPC on responds of albumin denaturation assay

It clearly indicates that the test drug NPC was effective in inhibiting heat induced albumin denaturation. Maximum percentage inhibition of about 46.49 ± 13.54 % was observed at 500 µg/ml, when compare to that of the Diclofenac sodium, a standard anti-inflammatory agent with the maximum inhibition
94.91 ± 3.37 % at the concentration of 100 µg/ml.

Biochemical analysis reveals

Neer peenisa choornam contain Calcium, Sulphate, Chloride, Starch, Ferrous iron, unsaturated compound and amino acid.

## 5) CONCLUSION

From this study, we can concluded that the test drug NEER PEENISA CHOORNAM possess significant anti-inflammatory property, so it is the most promising drug for Neer peenisam. This result can be further analysed in –vivo and more promising result may be expected.

## 6) ACKNOWLEDGEMENT

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