

Pharmacological Evaluation of Antiinflammatory Activity for Ethanolic Extract of *Ipomea Quamoclit* Linn., Leaves Against Egg Albumin Induced Paw Edema in Rats.

Ms. M. J. Sarah Ruth¹, Dr. Chintakunta Nagaraju²,
Mrs. V. Rajani, M. Pharm, (Ph. D) *

¹Sri Krishnadevaraya University College of Pharmaceutical Sciences, ²Academic consultant, Department of Biochemistry, Yogivemana university, ³Teaching faculty, Sri Krishnadevaraya University College of Pharmaceutical Sciences

msarah.j143@gmail.com, novahnagaraju@gmail.com, rajanivallepu390@gmail.com

Abstract:

Background:

In the 5th century B.C., Hippocrates created terms like "edema," which are still employed to describe inflammatory process. A favourable and crucial metabolic pathway, acute inflammation supports the body's ability to fight against pathogenic organisms as well as other foreign substances. The use of ethnomedicine has acquired magical and religious significance as humankind has progressed, and each human culture has created all its perceptions of health and disease. Plant-based therapeutic approaches are gaining popularity across the globe. In 2004 (Igoli et al.) Some of these plants have been found to have significant antimicrobial properties, fungicidal, anticarcinogenic, antidiuretic, antiinflammatory, and anti-diabetic properties.

Results:

The findings showed that, in both invitro and in vivo models, *Ipomoea quamoclit* L. ethanolic extract markedly and dose-dependently suppressed HRBC hemolysis, proteolysis, and egg albumin-induced paw edema. *Ipomoea quamoclit* L. leaf crude ethanolic extract displayed anti-inflammatory action towards egg albumin induced paw edema in rats. But more research work could support a new drug that works well in therapeutical facets for this field of medicine. Because of the great ability of ethanolic extract to fight back cell lysis and reduce edema size in small concentrations and especially in comparison to reference drug, our present research (indomethacin).

Conclusion:

The current study demonstrates that *Ipomoea quamoclit* L. ethanolic extract is safe to consume. Phytochemical *Ipomoea quamoclit* L.'s ethanolic leaf extract contains a phytoconstituents. So, it is possible to recommend the specimen for the synthesis of an antiinflammatory drug.

Keywords: *Ipomoea quamoclit*, Ethanolic-extract, Anti-inflammatory activity, Pharmacological evaluation, phytochemical screening, diclofenac-sodium, indomethacin.

Background:

Definition: Body's white blood cells as well as the substances they produce, known as INFLAMMATION, safeguard us from contagion by foreign invaders like pathogens.

The immunity frequently triggers an inflammatory response unreasonably. This is the scenario when there are autoimmune reactions. The body strikes its very own healthy tissue in retribution, acting as if they were abnormal or pathogenic. [1]

Types:

INFLAMMATION would be one of two things:

Acute (short lived): In few hours or even days, it disappears.

An advantageous and crucial process, acute inflammation supports the body's ability to fight against pathogenic organisms and some other foreign substances. The edema disappears once the cell has healed.

Its distinctive characteristics include the efflux of liquid and plasma proteins and the relocation of mainly leucocytes to the injured area. [2,3]

History:

Interpretations of this process can be found in ancient Greek and Egyptian texts, giving insight into the long and fascinating history of inflammatory process. In the 5th century B.C., Hippocrates created terms for inflammatory response, such as edema. He furthermore considered inflammatory process to be a type of cellular restoration at an early stage. However according to Roman author Aulus Celsus, who lived around 30 B.C. and 45 A.D., the four major signs of inflammation are skin irritability, heat, oedema, and frustration. The first three signs, according to what we currently know, are also most likely the outcome of the microvasculature's response to the inflammatory process. [3]

Therefore, as defence against damage or physical aggression, as it appears in individual. The idea contained in this explanation was recognized both by Galen (3rd century AD) and John Hunter (1794), an English physician from the 1800s. The papers by JARCHO (1970–1972) comprise an amazing wealth of historical data pertaining to a particular period. [4]

The earliest interpretation of proinflammatory cytokines could be found in Dutrochet's writings. He asserted that an individual's erythrocytes may leave the vasculature wall side to side and slowly move through the preparation's open space. The mucilaginous material was moving at a rate notably delayed than the blood that was already in motion. [5]

The conventional method of treating edema encourages recovering by employing treatment interventions that limit and even cease the inflammatory process, usually with the use of medications. By letting Physis to progress through the different phases of the inflammatory phases and method in a manner that encourages cure, the Tibb's action plan to inflammatory disease aims to maintain the physiological body in a position of stability, unity, and vigour. The biological, psychical, nostalgic, and religious forces that frame humankind must be honoured since they have a say in whether people are feeling happy or unhappy. [6]

Causes:

There are many things that cause inflammation. The following seem to be the most common characteristics:

- Pathogen-causing microorganisms, including such microbes, viral infections, or fungal spores

- Impacts of radiant energy or pollutants.
- Expressive injuries like abrasions or cause injury from foreign particles (like a claw in your palm).

An ailment or event that affects inflammation commonly lasts in "-itis" in its name. For illustration:

- The medical term for bladder swelling is cystitis.
- Bronchitis is an inflammatory reaction of the arterial system.
- Otitis media, an inflammatory condition of the middle ear
- Dermatitis: a skin inflammation state. [7]

Acute inflammation's morphological structure:

The release of exudates is a defining feature of an acute inflammatory reaction. Inflammatory mediators are usually present in an effusion, a high-protein edema fluid.

An organ-related disease that affects the heart, kidneys, malnutrition, as well as other organ systems can result in a transudate, which is simply a non-inflammatory edema.

Acute inflammatory consequences:

Positive outcomes:

- a) **Toxin dilution:** Toxins from bacteria and chemicals are diluted in exudate, which lowers their concentration at the inflammation site. Toxins are also removed from the site when secretions flow from the venous system through the skin and into the lymphatic vessels.
- b) **Defensive antibodies:** Exudation causes plasma proteins, including antibodies, to be present at the area of inflammation. As a result, antibodies made against the responsible organisms will respond and encourage microbial lysis by complement or phagocytosis.
- c) **Thrombin formation:** This slows the bacteria from spreading and improves leukocyte phagocytosis.
- d) **Plasma mediator systems** are delivered to the site of injury by the inflammatory process, including the supplement, blood clotting, anti - platelet, and kinin systems.
- e) **Cell nutrition:** As inflammatory exudates flow, they carry nutrients such as glucose, oxygen, and other essential minerals to help the vastly increased count meet their metabolic needs. Additionally, it eliminates their soluble wastages through lymph system.
- f) **Immune system stimulation:** Microbes and their pollutants are transported by secretions along the lymph vessels to local lymph nodes where they trigger an immune response with the producing antibodies and cells natural defence processes.

Negative outcomes:

- a) **Destruction of tissue:** Tissue cell death caused by inflammation may itself trigger more inflammation.
- b) **Swelling:** Inflammation-related swelling can have major mechanical impacts in some places. Instances involve acute meningitis and encephalopathy with impacts on elevated intracranial pressure and acute tonsillitis with intervention with respiration.
- c) **Irrational response:** The inflammatory responses observed in oversensitivity are an irrational response (i.e., exaggerated).

Chronic (long lasting): can last months or years, even after the first trigger is gone, conditions linked to the chronic inflammation which include cancer, diabetes, asthma, Alzheimer's disease.

Based on histopathologic characteristics, chronic inflammation can be subdivided into 2 types:

- a) Non-specific chronic inflammation: It is characterized by a dispersed collection of both lymphocytes and macrophages at the site of the injury, which is typically constructive and causes the growth of new fibrous tissue. For instance, chronic cholecystitis.
- b) Definition: Specific inflammation (granulomatous inflammation) Granulomas are what give granulomatous inflammation its name. A light microscope collection of epithelial cells is known as a granuloma. An altered microbe that resembles an epithelial cell is called an epithelioid cell (hence the name epithelioid). The epithelioid cells are capable of fusing with one another to create stromal cells. Despite the fact that a granuloma is essentially a grouping of 20 epithelioid cells, it also frequently contains stromal cells and is encircled by immune cells, occasionally with the addition of plasma cells. [8]

Inflammation-related symptoms:

- Skin irritation
- An inflamed potentially warm-to-the-touch
- Muscle pain
- Rigidity
- A flu
- Chill bumps

Inflammation that causes physical harm to the body and may be made worse by bacterial or fungal colonization. It could be limited, but in certain circumstances doing so results in irreversible. [9]

A healing plant:

Any plant that contains substances with medicinal benefits or that may be utilized as raw materials in the creation of drugs and therapies is regarded as a pharmacological activity. With the help of this definition, it is possible to distinguish between plants that are believed to have medicinal value but have not yet undergone a thorough research work and plants for which clinical effects and constituents have already been described observationally.

Native herbs are used in approximately 90% of traditional system of medicine recipe ideas and therapies, but this article will focus primarily on those who have been connected to disease prevention practices. In some cases, the line between therapy and diagnosis can be extremely thin. Take into account the fact that glomerular disorder can indeed be managed to avoid by attempting to treat mild hypertension elevations. [11]

Therapies made from plants are becoming more and more popular worldwide. [12] There were significant advancements in the therapeutical assessment of various plants for use in conventional medical processes thanks to recent research on medicinal herbs or medicines. A broad range of bioactive molecules or compounds, including tannins, terpenoids, alkaloids, and flavonoids, are found in herbal medicines. These substances determine the therapeutic potency of the plants, particularly their antimicrobial activities. The use of plant-derived remedies as an indigenous treatment in traditional medical systems has indeed been related to the introduction of plant-derived drugs in modern medicine. [13] Major antibacterial, antifungal, anticancer,

antidiuretic, anti-inflammatory, and antidiabetic characteristics were discovered in a few of these plants. [14,15,16,17,18]

Plant-based substances are becoming increasingly popular around the world. When compared to standard medicines, which work in an integrated or pro-biotic way with absolute minimum to no negative impacts on the body, medicinally able to prepare substances may work quickly, but they lead to serious complications that have a long-term negative impact on the human. Conventional medicine had also taken on magical and religious significance as humankind has evolved, so each civilization has established its own understandings of health and diseases. Several plants have been used in medical interventions such as Genuine Medicine in China, India, and Africa for over three thousand years. The large number of such plants have already been proven to have medicinal benefits by Western standards. [19]

Materials and methods:

Chemicals:

All chemicals used in this study are from Sri Krishnadevaraya University College of Pharmaceutical Sciences, Anantapur.

Collection of plant material:

Dr. B. Ravi Prasad Rao, Head of the Department of Botany, Sri Krishnadevaraya University, Ananthapuram, biologically identified and authenticated *Ipomoea quamoclit* leaves procured from agricultural land near Yemmiganur in the Kurnool district of Andhra Pradesh, India.

Extraction:

The extraction procedure was carried out using the Soxhlet apparatus. The Soxhlet extractor was invented by Ritter Von Soxhlet in 1879 (Soxhlet 1879), mainly for lipid extraction. It is, however, now commonly utilized to extract pharmacologically important elements from a wide range of herbal materials. [20,21]

Ipomoea quamoclit L. leaves were shade dried for 4 weeks before being ground into a powder form weighing 50 g. Ethanol was used as a solvent (200 ml). The process of extracting will last 8 hours at 60-80 degrees Celsius. The EIQ (Ethanol extract of *Ipomoea quamoclit*) extract was left to dry, and the dried mass was weighed and recorded. The dried mass obtained weighed nearly 2.5 g.

Methodology:

Phytochemical screening:

The plant extract (EIQ) was evaluated for the presence of preliminary phytochemical using the following accepted procedures.

Test for alkaloids:

- i. Hager's test: The test solution was treated by adding a few drops of Hager's reagent (saturated picric acid solution). The existence of alkaloids will be indicated by the formation of yellow precipitate. [22]

Meyer's test: Add 500 µl HCl and 3 drops Meyer's reagent to the extract. The existence of alkaloids is indicated by the formation of a creamy white precipitate. [23]

- ii. Test for tannins:

Lead acetate test: The extract was given a few droplets of lead acetate. A yellow precipitate formed, indicating the existence of tannins. [24]

iii. Test for flavonoids:

Ammonia test: Three drops of ammonium solution to the leaf extract. The appearance of a yellow colour indicates the existence of flavonoids.

Lead acetate test: Some few droplets of lead acetate were added to the leaf extract.

The appearance of yellow precipitate signifies the existence of flavonoids.

iv. Test for saponins:

Froth test: The sample was added with distilled water and thoroughly shaken to produce a stable, continual froth. The adding of olive oil results in the development of an emulsion, confirms the existence of saponins.

v. Test for phenols:

Upon dissolving 50 mg of the leaf extract in 5 ml of distilled water as well as adding some few droplets of neutral 5% ferric chloride solution, the formation of a dark green color was viewed positively for phenols.

vi. Test for cardiac glycosides:

The existence of cardiac glycosides is indicated by the formation of a brown ring at the intermediate layer following the introduction of 2 ml of glacial acetic acid containing one drop of ferric chloride solution, followed by the addition of 1 ml of concentrated sulfuric acid to 0.5 mg of extract prepared by diluting with 5 ml of water. [25]

vii. Test for terpenoids:

Add 200 μ l of chloroform and 300 μ l of concentrated H₂SO₄ to the leaf extract. The existence of terpenoids is indicated by the reddish-brown colour noted at the interaction. [26]

viii. Test for steroids:

2ml of acetic anhydride has been added to the leaf extract, preceded by 2ml of H₂SO₄. The colour was started changing from violet to blue or green, indicating that steroids were present. [27]

ix. Test for carbohydrates:

Benedict's test: Added a few drops of Benedict's reagent to the leaf extract (alkaline solution containing cupric citrate complex). This was boiled in a water bath. The existence of reddish-brown precipitate signifies the existence of carbohydrates.

Invitro methods:

Erythrocyte suspension:

- A healthy individual's whole blood was taken.
- Blood has been centrifuged for 5 minutes at 3000 rpm in anticoagulated microcentrifuge tube.
- Thoroughly washed with equal amounts of normal saline (0.9% NaCl).
- The blood sample was measured after centrifugation and reconstituted as a 10% (v/v) suspension with isotonic buffer solution (10 mM sodium phosphate buffer pH 7.4). [28,29]

a) Heat induced hemolysis:

- The mixture (2ml) contains of 1ml test group at varying concentrations (10,30,50,70,90) g/ml and 10% red blood cell suspension (1ml), with the control test tube containing only vehicle.

- The standard dose is 50mg Diclofenac sodium.
- By inversion, this mixture is mixed gently.
- All centrifuge supernatants are placed into a water bath at 60 degrees Celsius for 30 minutes.
- The tubes are allowed to cool under running water at the final moment of the incubation.
- The mixture and all samples were centrifuged for 5 minutes at 3000 rpm, and the absorption spectrum of the supernatants was measured at 560 nm.
- The observation is performed three times for each sample.
- The following formula is used to calculate the inhibitory activity of hemolysis:
- Percentage inhibition of haemolysis = $\frac{\text{Absorbance Control}-\text{Absorbance test}}{\text{Absorbance control}} \times 100$ [30,31]

b) Protein denaturation method:

An in vitro anti-arthritis assay was performed using Mizushima and Kobayashi's protein denaturation method (1968). The test mixture (5 ml) comprises 2 ml of leaf extract (0.01, 0.1, 1, 10, 100, 1000 g/ml), 0.2 ml of egg albumin, 2.8 ml of phosphate-buffered saline (PBS with pH 6.4), and 2 ml of the mixture. A similar amount of distilled water serves as a control. After incubating at 37 °C for 15 minutes in a BOD incubator, the mixture was warmed at 70 °C for 5 minutes. Upon cooling, their absorption spectra at 660 nm were taken by using vehicle as a basis for comparison.

The percentage of protein denaturation inhibitory activity was calculated using the formula below. [32]

$$\text{Inhibition} = \frac{\text{AC (660)} - \text{AT (660)}}{\text{AC (660)}}$$

AC = Absorbance of control solution, AT = Absorbance of the test sample.

Experimental animals:

For the entire study, healthy male Albino Wistar rats (body weight 100-150) have been used. These animals are housed in the animal house at SK University under recommended climatic conditions. All animal studies performed throughout this research project were approved by the Institutional Animal Ethics Committee (IAEC) and then were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) established by the Animal Welfare Division of the Government of India. [CPCSEA No. 1889/GO/Re/S/16/CPCSEA, IAEC/1/02/SKU/2022 Protocol]. These rats were brought from the Biogen laboratory animal facility in Bangalore [CPCSEA No: 971/bc/06/CPCSEA]. All animals were supplied with marketing pellet rat feed and given free access to water.

Egg albumin induced paw edema in rats:

Table:1

| Animal group | Description |
|--------------|--|
| Group-I | Normal control group received normal feed and water. |
| Group-II | Control group received vehicle |
| Group-III | Standard group received indomethacin 90% (10 mg/kg body weight) |
| Group-IV | Received EIQ (100 mg/kg, body weight for 10 days + on 10 th day after final dose, received egg albumin 1% 0.1 ml. |
| Group-V | Received EIQ (200 mg/kg. body weight) + received egg albumin 1% 0.1 ml on the day of experiment. |

Group I was given a placebo, while Group II was given the reference drug indomethacin (10 mg/kg body weight). EIQ (Ethanolic extract of *Ipomoea quamoclit*) was given to groups III and IV for ten days at concentrations of 100 and 200 mg/kg via stainless steel gavage needle. All rats in all groups received fresh undiluted egg albumin 1% 0.1 ml. Upon 1 hour, plethysmometer was used to observe edema formation in the groups for 5 hours. [33]

Parameters:

Volume of right hind paw:

The rats' right hind paw volumes were measured using a plethysmometer and the volume displacement principle.

1. Determination of V_t :

The right hind paw volume of the rats was measured using a plethysmometer at 1 hour intervals after an egg albumin injection for a duration of five hours.

2. Determination of V_0 :

The rats' right hind paw volume was measured using a plethysmometer instantly just before experiment based on volume displacement (zero time).

Results:

1. Preliminary phytochemical screening of ethanolic extract of *Ipomoea quamoclit* Linn.,

Table:2

| Tests | EIQ (Ethanolic extract of <i>Ipomoea quamoclit</i> (Present: (+), Absent: (-)) |
|-----------|--|
| Alkaloids | + |
| Tannins | + |

| | |
|--------------------|---|
| Flavonoids | + |
| Saponins | - |
| Phenols | + |
| Cardiac glycosides | + |
| Terpenoids | + |
| Steroids | - |
| Carbohydrates | - |

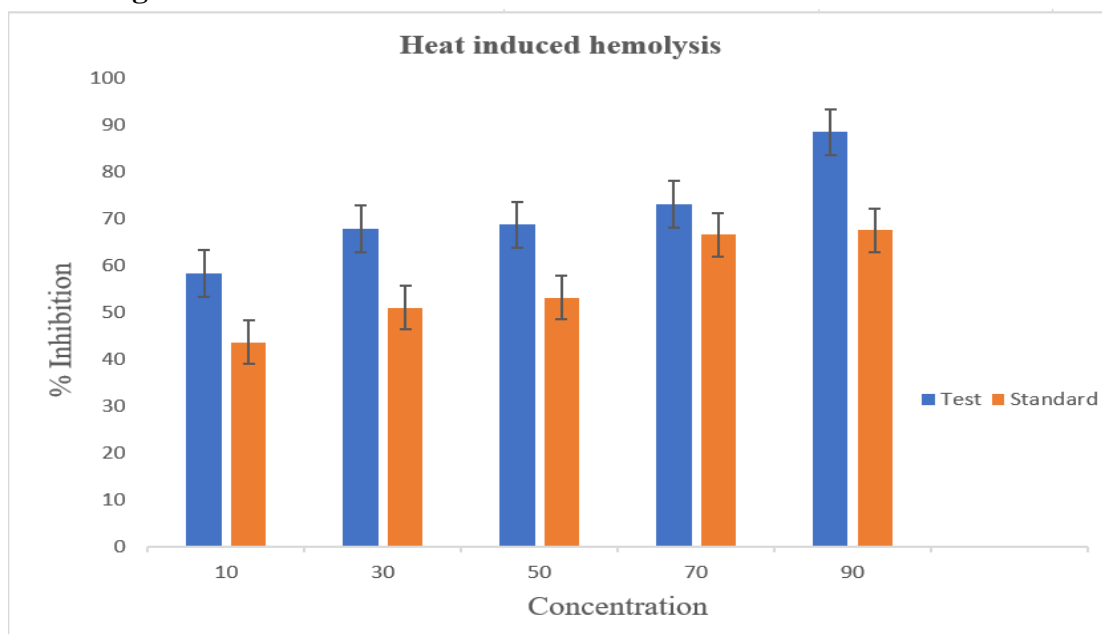
I. Invitro studies:

a) Heat induced hemolysis:

Table:3

| S. No | Concentration (µg/ml) | Percentage Inhibition (%) Mean ± SEM | |
|-------|-----------------------|---|--|
| | | Diclofenac sodium (Standard) | EIQ (Ethanolic extract of Ipomoea Quamoclit) |
| 1. | 10 | 0.655±0.00033 (43.65%) | 0.232±0.00033 (58.28%) |
| 2. | 30 | 0.674±0.00176 (50.99%) | 0.542±0.00033 (67.86%) |
| 3. | 50 | 0.945±0.00173 (53.12%) | 0.631±0.00176 (68.7%) |
| 4. | 70 | 0.988±0.00608 (66.57%) | 0.648±0.00173 (73.11%) |
| 5. | 90 | 1.136±0.00493 (67.51%) | 0.841±0.00202 (88.49%) |

Figure:1

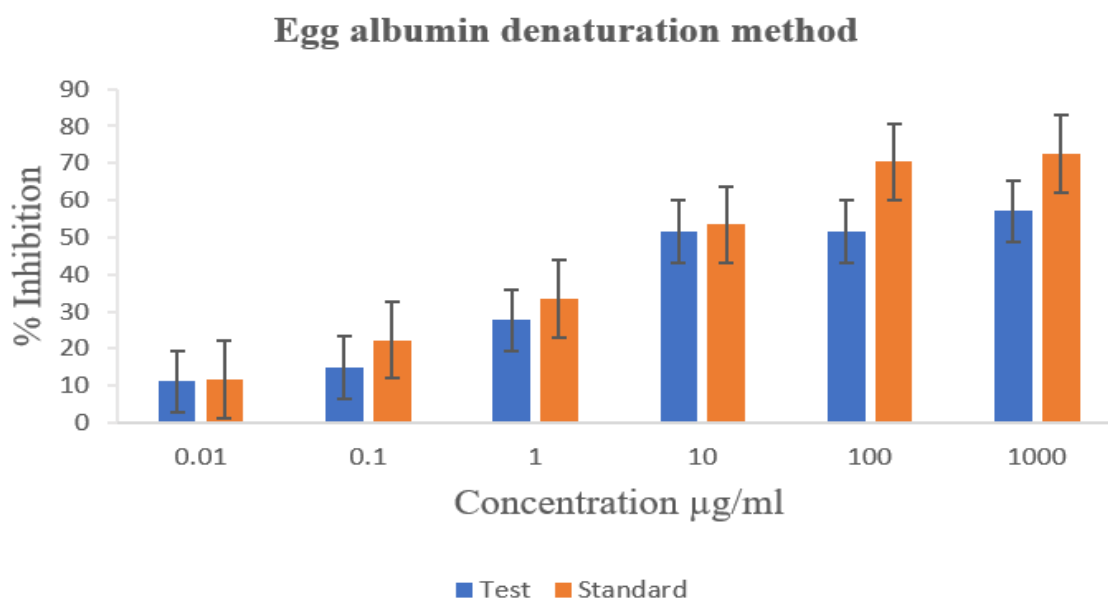


b) Egg albumin denaturation method:

Table:4

| S. No | Concentration (µg/ml) | Percentage Inhibition (%) Mean ± SEM | |
|-------|-----------------------|--|------------------------------|
| | | EIQ (Ethanolic extract of Ipomoea Quamoclit) | Diclofenac sodium (Standard) |
| 1. | 0.01 | 0.53±0.0043 (11.22%) | 0.52±0.0081 (11.72%) |
| 2. | 0.1 | 0.50±0.0060 (14.90%) | 0.46±0.0023 (22.27%) |
| 3. | 1 | 0.43±0.0046 (27.63%) | 0.39±0.0057 (33.5%) |
| 4. | 10 | 0.28±0.0012 (51.59%) | 0.27±0.0063 (53.43%) |
| 5. | 100 | 0.28±0.0008 (51.59%) | 0.17±0.0066 (70.35%) |
| 6. | 1000 | 0.25±0.0026 (57.11%) | 0.16±0.0049 (72.52%) |

Figure:2



II. In vivo studies:

Egg albumin induced paw edema in rats:

Table:5

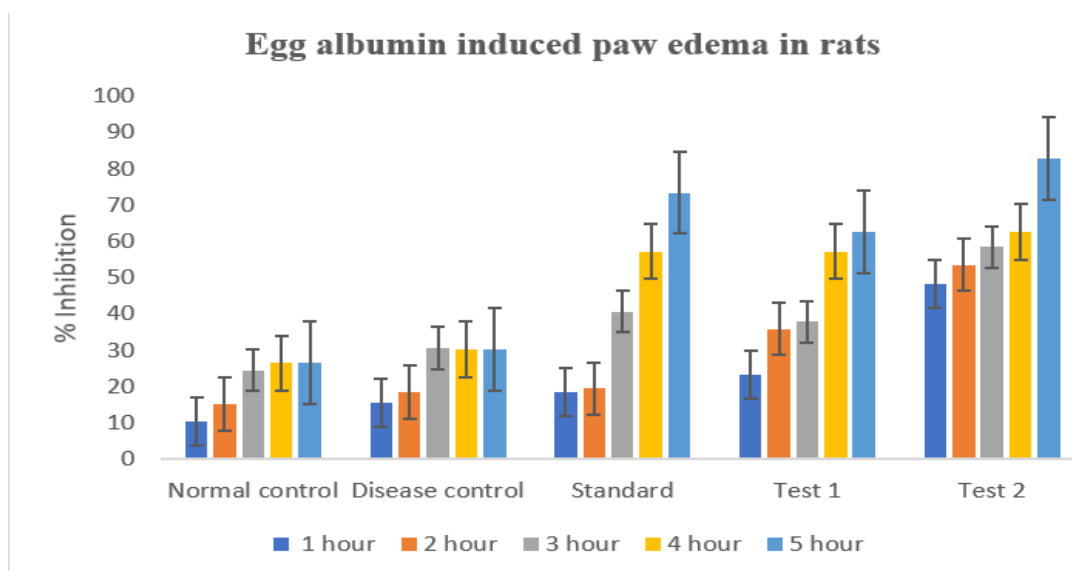
| Treatment | Dose | 1 hr | 2 hr | 3 hr | 4 hr | 5 hr |
|-----------------|------|---------------------|---------------------|---------------------|---------------------|---------------------|
| Normal control | | 1.75±0.05 (10.3) | 1.7±0.057 (15.2) | 1.5±0.057 (24.5) | 1.4±0 (26.5) | 1.4±0 (26.5) |
| Disease control | | 1.12±0.08 (15.4) | 1.11±0.07 (18.5) | 1.09±0.06 (30.6) | 1.07±0.06 (32.2) | 1.09±0.06 (30.4) |

| | | | | | | |
|----------------------|-----------|--------------------|---------------------|----------------------|----------------------|----------------------|
| Standard | 10 mg/kg | 1.04±0.07 (18.40) | 0.90±0.06 (19.50) | 0.63±0.07** (40.69) | 0.44±0.05*** (57.20) | 0.27±0.07*** (73.48) |
| T₁ | 100 mg/kg | 0.77±0.07* (23.14) | 0.71±0.04** (35.87) | 0.62±0.06** (37.87) | 0.44±0.05*** (57.14) | 0.41±0.06*** (62.62) |
| T₂ | 200 mg/kg | 0.59±0.06* (48.25) | 0.51±0.04** (53.55) | 0.44±0.05*** (58.40) | 0.39±0.04** (62.61) | 0.22±0.09*** (82.81) |

Reduction in edema significant at P<0.05 compared to control.

P<0.01, *P<0.001, values of edema are shown as mean ± SEM (n=5), values in parenthesis () are % inhibition of edema calculated relative to control.

Figure:3



Discussion:

Provided that the condition is involved among almost all diseases affecting both humans and animals, it has recently become the subject of significant scientific research on a global scale. Plant-based compounds used in the traditional treatment of diseases such as edema had indeed captured the attention of scientists in latest days because they are inexpensive and high therapeutic activities without any of the associated side effects with synthesized oral medications. [33]

The current study found that an ethanolic extract of Ipomoea quamoclit leaf extract significantly suppressed hemolysis in vitro and significantly reduced edema in albino wistar rats. In those other ways, these in vitro and in vivo studies were much more time-saving, adaptable, and comfortable. As a result, this finding has also discovered a few earlier allotments who proposed that perhaps the fragmentation of bio-membranes causes the generation of reactive oxygen species, which then in turn raises tissue injury. The analysis indicates that such an ethanolic extract of Ipomoea quamoclit L. could noticeably and dose dependently suppress HRBC hemolysis, protein denaturation, and egg albumin-induced paw edema in both invitro

and animal studies models. *Ipomoea quamoclit* L. leaf crude ethanolic extract displayed anti-inflammatory activity towards egg albumin induced paw edema in rats. This falls in line with several of the earlier discoveries. But more experimental studies could support a therapeutic agent that works well in therapeutical facets for this field of medicine. Because of the strong ability of ethanolic extract to resist cell lysis and reduce edema size in small concentrations and especially in comparison to reference drug, our current investigation (indomethacin). Therefore, it will be an extremely significant suspect for a drug candidate that can be isolated to treat the disease. Therefore, the required steps must be taken to take advantage of their biochemical pathway to this specific area of medical science. This plant has the potential to serve as a major contributor of lead for the effective treatment of diseases such as inflammation.

Conclusion:

Ipomoea quamoclit L. leaf extract contains a phytoconstituents that has the possibility to be employed in the creation of anti-inflammatory medications. *Ipomoea quamoclit* L. leaf extract tested in both vitro and in vivo for anti-inflammatory activity yields a modest degree of activity, Reduction in edema significant at $P < 0.05$ compared to control. $P < 0.01$, $P < 0.001$, values of edema are shown as mean \pm SEM (n=5), values in parenthesis () are % inhibition of edema calculated relative to control.

The current research demonstrates that *Ipomoea quamoclit* L.'s ethanolic extract has significant anti-inflammation activity. So, it is possible to recommend the sample for the preparation of an anti-inflammatory drug. The current research indicates that the sample might be useful for finding a novel anti-inflammatory medication. The sample's separation, purifying, and categorisation may be utilized in subsequent studies.

References:

1. Zelman, D. (2022). Inflammation. WebMD, .
2. Barhum, L. (2022). What Is Inflammation? Types, Causes, Symptoms, and Treatment. verywellhealth.
3. U. Ahmed, A. (2011). (PDF) An overview of inflammation: Mechanism and consequences. *Frontiers in Biology*, 10.1007/s11515-011-1123-9
4. DN, G. & E, S. Historical Perspectives - Inflammation and the Microcirculation - NCBI Bookshelf.
5. E silva, M. Rochae. A Brief History of Inflammation | SpringerLink.50/1, 6-25. https://doi.org/10.1007/978-3-642-66888-3_2
6. Ley, K. (2001). History of Inflammation Research. ResearchGate, , 1-10. 10.1007/978-1-4614-7512-5_1
7. Mayer, L. & Bhikha, R. (2013). Overview-and-historical-significance-of inflammation. tibt.
8. Cologne, (2010). What is an inflammation? - InformedHealth.org - NCBI Bookshelf.
9. What is Acute Inflammation: Symptoms, Causes and Treatment - CBD Web.
10. Altameemi, A. & Sajid Mohammed, Z. (2019). [PDF] inflammation. ResearchGate.
11. Sofowora, A., Ogunbodede, E. & Onayade, A. (2013). The Role and Place of Medicinal Plants in the Strategies for Disease Prevention. *Afr J Tradit Complement Altern Med.*, 10(5), 210–229. 10.4314/ajtcam.v10i5.2

12. Sofowora A. *Medicinal Plants and Traditional Medicine in Africa*. 3rd edn. Ibadan: Spectrum Books; 2008.
13. Ahmed Dar, R., Shahnawaz, M. & Hassan Qazi, P. (2017). General overview of medicinal plants: A review. *The Journal of Phytopharmacology*, 2017; 6(6): 349-351(2320-480X).
14. Igoli, J. O., Igwue, I. C. & Igoli, N. P. (2004). Traditional Medicinal Practices Among the Igede People of Nigeria. *Journal of Herbs, Spices & Medicinal Plants*, 10. https://doi.org/10.1300/J044v10n04_01
15. Sule, W., Okonko, I. & Ojezele, M. (2010). (PDF) In vitro Antifungal Activity of Senna alata Linn. Crude Leaf Extract. *Research Journal of Biological Sciences*, (1815-8846), 275-284. 10.3923/rjbsci.2010.275.284
16. Sy, T., LamuF, W., & As, R. (2012). ACUTE TOXICITY, PHYTOCHEMISTRY AND ANTIBACTERIAL ACTIVITY OF AQUEOUS AND ETHANOLIC LEAF EXTRACTS OF CASSIA ALATA LINN.
17. Oladeji, S. (2016). Spectrophotometric Analysis of Phenolic Compounds in Senna Alata | OLADEJI | *American Journal of Advanced Scientific Research*. *American Journal of Advanced Scientific Research*, 3(2), 246-253.
18. SM, M., Babangida Ali, D. & BZ, M. (2010). (PDF) CUTANEOUS WOUND HEALING ACTIVITY OF THE ETHANOLIC EXTRACTS OF THE LEAF OF SENNA ALATA L. (FABACEAE). *ResearchGate*, 2.
19. Garg, A. & Faheem, M. Role of Medicinal Plant in Human Health Disease | *Insight Medical Publishing*. *Asian Journal of Plant Science and Research*.
20. Kasiramar, Gopalasatheeskumar & k, Gopalasatheeskumar. (2019). significant role of soxhlet extraction process in phytochemical research. 7.
21. Bhandary, Satheesh & Kumari, Suchetha & Bhat, Vadisha & Sherly, Sharmila & Bekal, Mahesh. (2012). Preliminary phytochemical screening of various extracts of Punica granatum peel, whole fruit and seeds. *Nitte University Journal of Health Science*. 2. 10.1055/s-0040-17036
22. Kollakondan Muthaiya Maria John et al., Phytochemical screening and antioxidant activity of different solvent extracts from Strychnos minor Dennst leaves. *Asian Pac J Trop Dis* 2015; 5(3): 204-209. DOI: 10.1016/S2222-1808(14)60654-X.
23. Muhammad Dawood Shah, M. Amzad Hossain, Total flavonoids content and biochemical screening of the leaves of tropical endemic medicinal plant Merremia borneensis, *Arabian Journal of Chemistry*, Volume 7, Issue 6, 2014, DOI: <https://doi.org/10.1016/j.arabjc.2010.12.033>
24. Rahman Gul et al., Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from Ephedra intermedia Indigenous to Balochistan. *The Scientific World Journal* / 2017. DOI: <https://doi.org/10.1155/2017/5873648>.
25. Zakia Khanam et al., Phytochemical screening and antimicrobial activity of root and stem extracts of wild Eurycoma longifolia Jack (Tongkat Ali). *journal of king saud university-science* (2015) 27, 23-30. <http://dx.doi.org/10.1016/j.jksus.2014.04.006>.
26. Sharma, et al.: Antimicrobial and Phytochemical Evaluation of Jatropha curcas Linn. *Pharmacognosy Journal* | July-August 2012 | Vol 4 | Issue 30. DOI: 10.5530/pj.2012.30.7

27. Juvekar, Archana & Sakat, Sachin & Wankhede, Sangeeta & Juvekar, M & Gambhire, M.. (2009). In vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. *Planta Medica - PLANTA MED.* 75. 10.1055/s-0029-1234983.
28. Sen, N., Bulbul, L., Hussain, F., & Amin, M. T. (2016). Assessment of thrombolytic, membrane stabilizing potential and total phenolic content of *Typha elephantina* Roxb. *Journal of Medicinal Plants Research*, 10(38), 669-675.
29. Shinde, U. A., Kulkarni, K. R., Phadke, A. S., Nair, A. M., Mungantiwar, A. A., Dikshit, V. J., & Saraf, M. N. (1999). Mast cell stabilizing and lipoxygenase inhibitory activity of *Cedrus deodara* (Roxb.) Loud. wood oil. *Indian journal of experimental biology*, 37(3), 258–261.
30. Moualek, Idir & Aiche, Ghenima & Guechaoui, Nadjat & Lahcene, Souad & Houali, Karim. (2016). Antioxidant and anti-inflammatory activities of *Arbutus unedo* aqueous extract. *Asian Pacific Journal of Tropical Biomedicine.* 6. 10.1016/j.apjtb.2016.09.002.
31. Mizushima, Y. & Kobayashi, M. (1968). Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. *Journal of Pharmacy and Pharmacology*, 20(3), 169–173.
<https://doi.org/10.1111/j.2042-7158.1968.tb09718.x>
32. Anosike, Chioma & Obidoa, Onyechi & Ezeanyika, Lawrence. (2012). The anti-inflammatory activity of garden egg (*Solanum aethiopicum*) on egg albumin—induced oedema and granuloma tissue formation in rats. *Asian Pacific journal of tropical medicine.* 5. 62-6. 10.1016/S1995-7645(11)60247-2.
33. Okwu, Donatus & U. Ph. D, Nnamdi. (2012). Exotic multifaceted medicinal plants of drugs and pharmaceutical industries. *African Journal of Biotechnology.* 8.