



PHYTOCHEMICAL ANALYSIS AND ANTI-INFLAMMATORY ACTIVITY OF THE EXTRACT OF LEAVES OF *TINOSPORA CORDIFOLIA*

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Abstract:

Background: *Tinospora cordifolia* is a well-known medicinal plant that is used to treat a variety of illnesses in traditional remedies. The common names Amrita and Guduchi are members of the Menispermaceae family.

Aim: Since *Tinospora cordifolia* is a plant that has not received enough evaluation, we intend to carry out anti-inflammatory activities. extraction of the plant material by a maceration procedure using different polarity solutions. Extraction of the material using procedure of distillation and evaporation followed by pharmacological and phytochemical analysis using a variety of chemical tests. **Methods:** Our study's methodology involves using an Oedema model caused by egg white to measure the anti-inflammatory efficacy. **Results:** It has been used to cure a wide range of illnesses, including diabetes, leprosy, urinary tract problems, diarrhea, and skin conditions. It is regarded as an important medicinal plant in the Indian system of medicine (ISM). **Conclusion:** Alkaloids, terpenoids, lignins, steroids, and other chemical compounds have been found in the plant, which support *Tinospora cordifolia*'s phytochemistry and pharmacological efficacy.

Key Words: *Tinospora cordifolia*, anti-inflammatory, egg white, edema, leprosy

INTRODUCTION:

Herbal introduction: Plants, illness, and life all come into play when individuals are born. Early humans started learning about illnesses and breastfeeding. Although there is no proof that people take synthetic medications for illnesses that occur before hospital admission, they are attempting to use easily accessible resources. They are often found in plants and animals that inhabit the environment¹. When they began utilizing plants, they discovered that the majority of them were either harmful or medicines, or else similar to other plants. The foundation of traditional Indian health care systems is medicinal herbs. Research on pharmacology has demonstrated that the usefulness of medicinal plants can serve as a possible source of chemicals with biological activity². Herbal phytochemical function is a key component in the research and creation of pharmaceuticals. *Tinospora cordifolia* commonly named as “**Guduchi**” is known for its immense application in the treatment of various diseases in the traditional ayurvedic literature. *Tinospora cordifolia* has an importance in traditional Ayurvedic medicine used for ages in the treatment of fever, pain, asthma, jaundice, dysentery, bone fracture, skin disease, chronic diarrhoea, cancer, poisonous insect, snake bite, eye disorders³.

Inflammation

Living mammalian tissues respond to harm locally by undergoing inflammation. It is the body's defensive mechanism to get rid of or stop harmful substances from spreading. An inflammatory response might include a number of different components that can aggravate tissue damage and cause related symptoms⁴. These elements of inflammation include the development of granulomas, oedema, and leukocyte infiltration¹¹. A synergy between several inflammatory mediators that increase vascular permeability and/or the mediators that increase blood flow causes oedema development in the paw⁵.

MATERIALS AND METHODS

Materials required:

Glassware Soxhlet apparatus, Desiccator, Test tubes, Glass wine syringe, Petri dish, Distillation set, Watch glass, Measuring cylinder.

Solvents: Methanol, Petroleum ether, Water.

Chemicals: Plant material, Egg white, Indomethacine

METHODOLOGY:

Plant Collection

Leaves of *Tinospora cordifolia* were collected from local Vangapally village, in the month of september, 2023.

Shade drying

Leaves of *Tinospora cordifolia* were shade dried to avoid the evaporation of volatile active constituents.

Method chosen for the extraction

Continuous Hot Percolation Process The continuous Hot Percolation process is used to remove the leaves. The most widely used example of a semi-continuous process for extracting lipids from food is the one Soxhlet (1879)

described⁶. The Soxhlet method involves repeatedly washing (percolating) an organic solvent—typically hexane or petroleum ether—under reflux in specialized glassware in order to extract oil and fat from solid material⁷.

Extraction Process of *Tinospora cordifolia*

A Soxhlet apparatus containing 200 gm of powdered *Tinospora cordifolia* was used to extract the powder using 2-2.5 liters of petroleum ether at 40 C with continuous hot percolation. The extract underwent distillation, was kept on desiccators, and the yield value as a percentage was calculated. Depending on the polarity of each organic solvent, such as methanol, petroleum ether, and water, the same mark was repeated. The extracts' yield percentage was calculated⁸.

Phytochemical screening: The phytochemical tests was choosen by basic chemical test .

Experimental animal

For this investigation, 20±5 g of inbred Swiss albino mice of either sex that were two months old were employed. The mice came from the breed colony's stock. In the animal home, they were kept at 23±1°C with a relative humidity of 55±55% and a light/dark cycle of 12 hours⁹. Throughout the trial, mice were given a commercial pellet meal and unlimited access to water. The animals were brought into the lab at least one hour prior to the experiment's commencement¹⁰. The Institution of Animal Ethics Committee (IAEC) granted clearance for all animal procedures, which were carried out in compliance with guidelines for the responsible handling and application of laboratory animals. The mice's treatment is shown in Fig. 1.

Acute toxicity study: utilizing albino mice (n = 6), of either sex, chosen at random for the acute toxicity investigation, an acute oral toxicity study utilizing a methanolic extract of *Tinospora cordifolia* was conducted in accordance with OECD-423 criteria (acute toxic class technique). Before administering the mice, animals undergo a fasting period of one night without food and just water¹¹. The animals should be weighed and given the test material after the fasting period. Food may be delayed for an additional period of time after the drug has been delivered. Choose from one of four predetermined dose levels—5, 50, 300, or 2000 mg/kg body weight—to utilize as the first dosage. When data indicates that death is unlikely to occur at the maximum initial dosage level (2000 mg/kg body weight), then exceptionally, and only when justified by specific regulatory needs, the use of additional upper dose level of 3500 mg/kg and 5000 mg/kg body weight may be considered¹².



Fig: No: 1. Photos of Treated mice with different dose of the plant extracts

PHARMACOLOGICAL ACTIVITY

EGG ALBUMIN INDUCED INFLAMMATION

Egg albumin (0.1 mL, 1% in normal saline) was injected into the subplantar tissue of the right hind paw of mice to cause inflammation⁵¹. The injected paw's linear circumference was measured before to, and 0.5, 1, 2, 3, 4, and 5 hours following the phlogistic agent's administration¹³. Mice that had been starved for 24 hours were given the leaf extract (250 mg/kg i.p.) one hour prior to the induction of inflammation. distilled water (10 mL/kg) was given orally to the control group. The difference in paw circumference between the control group and those that were given the phlogistic drug 0.5, 1, 2, 3, 4, and 5 hours later was used to measure edema, or inflammation. Vernier calipers were used to measure the average (mean) edema¹⁴.

Statistical analysis

Data were expressed as the mean values $\pm$ standard deviation (S.D.) for each measurement. The data were also analyzed by one-way analysis of variance (one-way ANOVA). Results with $P < 0.05$ were considered significant.

RESULTS:

Preliminary Phytochemical

Tinospora cordifolia methanolic extracts include phytoconstituents such as alkaloids, carbohydrates, glycosides, phytosterol, saponins, tannins, proteins, flavonoids, and diterpenes, according to the results of the preliminary phytochemical screening.

Acute toxicity studies

For further pharmacological screening, the MEAS extracts were deemed safe and non-toxic as they did not exhibit any toxic symptoms or death in rats up to a dosage level of 5000 mg/kg body weight in the acute toxicity trials.

Table No. 1. Experimental protocol for the Determination of acute oral toxicity (LD₅₀) of methanolic extract of *Tinospora cordifolia*

Name of the study	Acute toxicity
Guideline followed	OECD 423 method-acute toxic class method
Animals	Healthy young adult Swiss albino mice, nulliparous, non-pregnant
Body weight	20 $\pm$ 5 g
Sex	Either sex
Administration of dose and volume	100 and 200 mg/kg body weight, single dose in 0.5 mL

Number of groups and animals	9 groups and 6 animals in each group.
Route of administration	Oral by using mice oral feeding needle
Vehicle	Distilled water

Table No.2. Study period and observation parameters for acute toxicity

Initial once observation	First 30 min and periodically 24 hr
Special attention	First 1-4 hr after drug administration
Long term observation	Up to 14 days
Direct observation parameters	Tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma.
Additional observation parameters	Skin and fur, eyes and mucous membrane, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern etc.

PHARMACOLOGICAL ACTIVITY

Table No.3. Acute toxicity studies of *Tinospora cordifolia* leaves

S.No	Treatment	Signs of Toxicity	Onset of toxicity	Weight variation	Duration of observation
1	METC (2000mg/kg)	Observed	After 20 hrs	5g	14 days
2	METC(4000mg/kg)	Observed	After 20 hrs	5g	14 days
3	METC(5000mg/kg)	Observed	After 20 hrs	5-10g	Till animals are alive

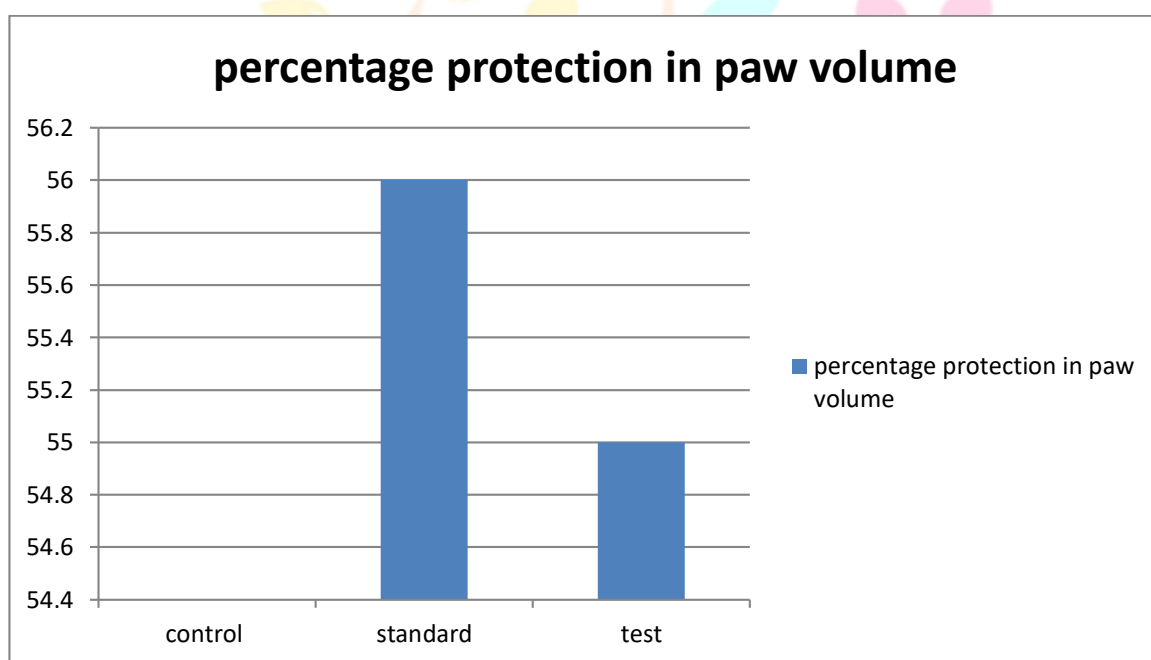
ANTI-INFLAMMATORY ACTIVITY

The anti-inflammatory potential of the methanolic extract of leaves of *Tinospora cordifolia* was investigated using egg white induced hind paw oedema method¹⁵. The results of methanolic extract of leaves of *Tinospora cordifolia* in egg white induced hind paw oedema were presented in Table 10.3. The results revealed that the methanolic extract of leaves of *Tinospora cordifolia* at 300 mgkg⁻¹ exhibited 55.80% inhibition; while idomethacin showed 56.09% (Table 10.3).

Table.No.4. Anti-inflammatory activity of methanolic extract of leaves of *Tinospora cordifolia* against egg white induced paw oedema in albino Wistar rats.

Treatment	% Increase in paw volume Mean $\pm$ S.E (n = 6) Post insult time of assay (min)					% Inhibition in paw vol.
	0	60	120	180	240	
Control	19.53 $\pm$ 1.20	81.83 $\pm$ 5.22	88.93 $\pm$ 3.92	95.20 $\pm$ 7.7	99.03 $\pm$ 7.21	-
METC (300 mg kg ⁻¹)	19.28 $\pm$ 0.83	70.38 $\pm$ 4.73	63.2 $\pm$ 2.50	58.8* $\pm$ 3.83	55.8 $\pm$ 2.81	55.8
Indomethacin (4 mg/kg)	14.2 $\pm$ 0.88	33.5 $\pm$ 1.83	38.9 $\pm$ 2.81	41.8* $\pm$ 3.2	58.82 $\pm$ 2.90	56.09

*p< 0.001 Vs Control by student's 't' test. MEAS: Methanolic extract of leaves of *Tinospora cordifolia*

**Figure No :2:** Anti-inflammatory activity of methanolic extract of leaves of *Tinospora cordifolia* against egg white induced paw oedema in albino Wistar rats.

DISCUSSION

Preliminary phytochemical

The results of a preliminary phytochemical screening indicated that the methanolic extract of *Tinospora cordifolia* exhibited a positive response to Alkaloids, Tannins, Flavonoids, Carbohydrates, Lignins, and Proteins. These constituents are accountable for various pharmacological activities, such as wound healing, hepatoprotective activity, and antidiarrheal and triterpene activity. Flavonoids, on the other hand, are responsible for neuro pharmacological and CNS depressants, and anti-inflammatory activity^{15,16}.

Acute toxicity

The extracts of METC were deemed safe and non-toxic for additional pharmacological screening because they did not exhibit any toxic symptoms or mortality in rats up to the dose level of 5000 mg/kg, 4000 mg/kg, or 3000 mg/kg body weight in the acute toxicity studies.

Anti-inflammatory

The extract also prevented the production of histamine and 5-HT, two mediators produced by egg albumin, which shows that it can reduce inflammation¹⁹. It also prevented egg albumin-induced oedema. It has been proposed that a number of inflammatory mediators, including complement, histamine, kinins, prostaglandins, and pro-inflammatory cytokines, are involved in the process of inflammation¹⁷. ASA, a cyclooxygenase inhibitor, on the other hand, dramatically decreased the edema caused by egg albumin. It has been revealed that plants' anti-inflammatory properties include flavonoids⁵³. It has been discovered that the extract contains these. It is believed that the methanolic extract of *Tinospora cordifolia* leaves inhibits at least some of these mediators. Based on its pathophysiology, inflammation may be classified into three categories: allergic, nonspecific, and infectious inflammation¹⁸. The following non-specific animal models are typically utilized in research of anti-inflammatory drugs: rats with paw Oedema caused by carrageenan, egg albumin, or histamine; mice with ear Oedema generated by xylene; mice with capillary permeability; and rats with cotton pellet granuloma⁵⁵. Therefore, the cotton pellet granuloma in rats is a great model of chronic inflammation that was chosen to study chronic inflammation, which consists of proliferative and transudate phases⁵⁶. Using the cotton pellet granuloma experiment, inflammatory response may be easily identified by granuloma development, extravasations, and different biochemical exudates. There is a strong correlation between the quantity of granulomatous tissue development and the dry weight of the cotton pellet implanted²⁰. Oedema, which arises following carrageen in inflammation, is a biphasic event²¹. MEVN may decrease the proliferative stages of inflammation by reducing the dry weights of implanted cotton pellets. Serotonin and histamine release are thought to be responsible for the first stage.

CONCLUSION:

Since traditional medicines have been utilized for thousands of years as natural medicinal cures across the world, it is generally acknowledged that a variety of ingredients contribute to their effectiveness. The outcome of this investigation suggested that METC has anti-inflammatory properties and that using it is safe²². The traditional usage of for therapy is supported by pharmacological data from these pharmacological actions. *Tinospora cordifolia* Steroids, triterpenoids, flavanoids, tannins, reducing sugar, and saponins are all detected by screening leaves²³. This extract's anti-oedematous properties might be attributed to the steroids, alkaloids, and triterpenoids found in it. Therefore, more research is necessary to fractionate, purify, and identify the active principle or principles that precede this extract as well as to precisely comprehend the mechanism of action behind the methanolic extract of *Tinospora cordifolia* leaves' anti-inflammatory properties.

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