

## Anti-Inflammatory Effect of Acetone, Methanolic and Aqueous Extracts of Traditional Herbal Drug Evaluated Using Cotton Pellet Method

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### ABSTRACT

The anti-inflammatory activity of a traditional antipyretic polyherbal drug was assessed by preparing its acetone, methanolic and aqueous extracts using cotton pellet method on rats. No mortality or toxic symptom was observed up to the dose of 1000 mg/kg during acute toxicity studies while flavonoids, tannins and carbohydrates were found present in all the extracts. The test drug exhibited highly significant anti-inflammatory effect in case of all extracts at 600 mg/kg dose which was close to that of the standard drug Indomethacin. Slightly lower impact was observed when using test drug dose of 400 mg/kg. Among the three extracts, the methanolic one exhibited the highest inhibition of granulation tissue formation, the aqueous extract having a slightly lower impact while the lowest effect was observed in case of the Acetone extract.

**Key words** –Traditional, Aqueous, methanolic, acetone extracts, sub-acute inflammation, toxicity

### INTRODUCTION

Non-steroidal anti-inflammatory medicines are a class of drugs that provide analgesic, antipyretic and anti-inflammatory effects. NSAIDs are usually indicated for the treatment of acute or chronic conditions where pain and inflammation are present, although their usage has been associated with some contra-indications <sup>1</sup>. At the same time, there are many medicinal plants which have been used since ancient times in the form of formulation for the treatment of pain, inflammation and fever as mentioned in the ancient literature Charak Samhita <sup>2</sup>.

A traditional polyherbal drug consisting of equal amounts of stems of Guduchi (*Tinospora cordifolia* Willd), fruits of Amlaki (*Emblica officinalis* Gaertn) and rhizomes of Mustak (*Cyperus rotundus* Linn) has been used for treatment of fever, pain & inflammation as indicated in the review of Ayurvedic literature <sup>2</sup>.

*Emblica officinalis* Gaertn (family Euphorbiaceae), locally known as “Amlaki” or “Amla” is a small or medium sized deciduous tree found throughout India. Dried fruit of the plant is brown to blackish brown in colour with characteristic odour and sour and astringent taste. Its fruits are a rich source of Vitamin C and also contain tannin, ellagic acid, gallic acid and phyllembins. They are used in cases of diabetes, anaemia, peptic ulcer, inflammation, skin diseases and cardiac problems. The fruit juice has also reported lipid lowering and anti-atherosclerotic effect <sup>3</sup>.

*Tinospora cordifolia* (family Menispermaceae) or “Guduchi” is a glabrous climbing shrub typically found growing in deciduous and dry forests throughout India. The succulent stem is creamy white to grey in color, with

deep clefts spotted with lenticels often giving out aerial roots. The principal constituents of its stem are Tinosporin, Tinocordiside, Tinocordifolioside, Cordioside and alkaloids like Berberine & Palmatine. This plant is used in Ayurvedic practice for treatment of various ailments like leprosy, fever, asthma, jaundice, diabetes, skin infections, diarrhoea and dysentery. It is generally considered as a rejuvenator and diuretic <sup>3,4,5</sup>.

Purple nutsedge or *Cyperus rotundus* Linn (family Cyperaceae) known locally as “Mustak” is a perennial weed. The plant is indigenous to India but is now spread in tropical, subtropical and temperate regions. The rhizomes are bluntly conical and vary in size and thickness, crowned with the remains of stem and leaves forming a scaly covering, dark brown or black externally, creamish-yellow internally and have pleasant odour. The rhizome contains polyphenols like Cyperone, Cyperenone and Cyperene, carbohydrates like D-glucose and D-fructose. In Indian System of Medicine, the rhizome of this plant is recommended for use in different clinical conditions including fever and arthritis. The rhizomes are cooling, nervine tonic, and diuretic and traditionally used to treat diarrhoea, dysentery, leprosy, bronchitis and blood disorders. The rhizome is reported to also possess analgesic, anti-inflammatory and antipyretic activity <sup>3,6,7</sup>.

The test formulation has been used since ancient times for treatment of chronic type of inflammatory diseases. The present study evaluates the toxicity and anti-inflammatory property of the various extracts of the test drug using the standard cotton pellet method on rats after getting significant results regarding its antipyretic effect.

## MATERIAL AND METHODS

The chemical analysis and experimental study was done in the laboratory and CPCSEA registered animal facility of Dravyaguna department of the Institute of Post Graduate Ayurvedic Education & Research, Kolkata.

**Collection & Identification of Plant material:** The fresh fruits of *Emblica officinalis*, stems of *Tinospora cordifolia* and rhizomes of *Cyperus rotundus* were purchased from crude drug supplier of the pharmacy of S.V.S.P. Hospital, Kolkata and shade dried. These samples were authenticated by the Research Officer at Botanical Survey of India, Shibpur, Howrah (Ref No-BSI/CNH/AD/Tech/2010, Date- 21.07.2010). The herbarium of the above mentioned plants are stored in the museum of Dravyaguna Department of IPGAER, Kolkata, India.

**Chemicals:** Chemicals for phytochemical screening and standard reagents were purchased from Merck Specialities Pvt. Ltd, Mumbai and. All chemicals used were of analytical grade.

**Animals:** All the experimental studies were carried out under suitable conditions in the animal house of IPGAER which is registered with the Committee for the Purpose of Control & Supervision of Experiments on Animals (CPCSEA), Govt of India bearing Reg. No-1180/ac/08/CPCSEA. Before undertaking experimental work, due permissions were obtained from the Institutional Animal Ethical Committee. Wister Rats (120-130 gm) and Swiss albino mice (20-30 gm) were used for the acute toxicity study. All the animals were procured from a reputed animal supplier, M/s Ghosh Scientific, Kolkata. All the animals used for this experiment were housed in polypropylene cages and maintained under environmentally controlled room provided with a 12: 12 hr light and dark cycle for each 24hr period at a temperature of approximately 25°C. They were fed with standard pellet diet and water *ad libitum*.

**Preparation of the Extraction of Test drug:** All the crude drugs were washed to remove extraneous matters such as dirt, foreign matter and adulterants, sun dried and crushed to particle size of 40 meshes. The powdered fruits of *Emblica officinalis*, stems of *Tinospora cordifolia* and rhizomes of *Cyperus rotundus* were mixed in equal proportion to prepare the Test Drug. The test drug was subsequently extracted sequentially in Petroleum ether (60°- 80°), Chloroform, Acetone, Methanol and Water using a Soxhlet Apparatus for 48 hours. The extracts obtained were filtered, concentrated in a rotary evaporator

and finally stored in refrigerator for further analysis and experimentation<sup>8</sup>.

**Physiochemical Analysis of test drug:** The macroscopic and microscopic study of the test drug powder was performed using the compound microscope<sup>9</sup>. Physiochemical parameters such as extractive value, moisture content, acid insoluble ash, water soluble ash and total ash content of the powdered test drug were evaluated according to the standard steps described in the Ayurvedic Pharmacopoeia of Department of AYUSH, Govt. of India.

**Phytochemical screening:** Preliminary phytochemical screening of the different constituents like alkaloids, flavonoids, tannins, carbohydrates, glycosides, saponins, fats and oils, proteins and amino acids was performed following standard procedures<sup>10,11</sup>.

## EXPERIMENTAL METHODS

**Acute toxicity study:** Acute oral toxicity study was carried out according to OECD guidelines 423. The animals of both sexes were selected by random sampling technique and divided into 5 groups of 3 animals each. A single oral dose (200, 400mg, 600mg, 800mg and 1000mg/kg) of each extract was administered orally at the dose level up to 1000mg/kg body weight. The animal groups were observed for appearance of toxic symptoms including behavioural changes, locomotion, muscle spasm, loss of righting reflex, tremor, convulsions and mortality for 24 hrs and further supervised for a period of 14 days for occurrence of toxic symptoms and mortality<sup>12</sup>.

**Cotton pellet induced granuloma formation:** The acclimatized rats in both sexes were divided in to eight groups each having six animals. Sterilized cotton pellets weighing 10 mg made from no.1 dental cotton rolls were implanted subcutaneously under light ether anaesthesia. Two pellets were implanted in each animal, one in each groin, and the edges of cut were stitched. The test drug in 2 different dosages (400mg/kg and 600mg/kg body weight) of each extract - aqueous, methanolic and acetone was administered orally for four consecutive days. The group A served as control (10 ml/kg Normal saline) while the group B which was kept as standard was given 2.5 mg/kg body weight Indomethacin every day for four consecutive days. The first dose of each sample was given immediately after the implantation of pellets. On the fifth day, pellets were dissected out, freed from extraneous tissues, dried overnight in a hot-air oven at 60°C and weight of each granuloma was then determined. The difference between the initial weight and the final

Table1: Results of physiochemical and phytochemical analysis

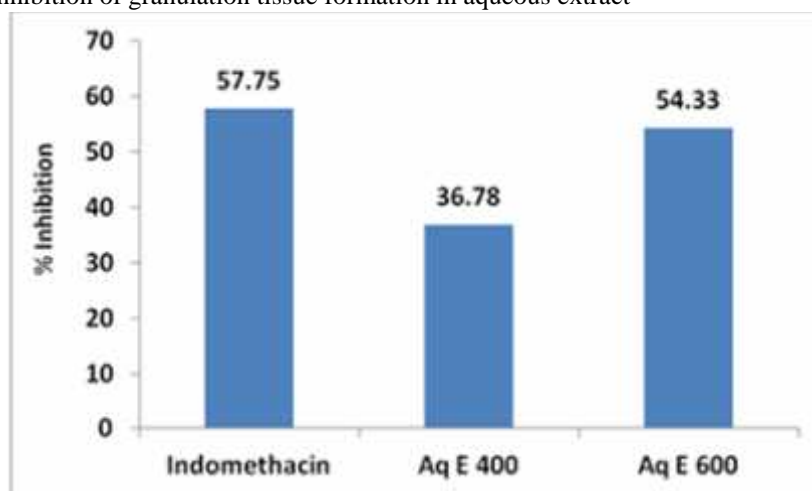
Name of test	Aqueous extract	Methanolic extract	Acetone extract
Extractive value	3.57	7.01	3.75
Alkaloid	Present	Absent	Absent
Flavonoid	Present	Present	Present
Saponin	Present	Present	Absent
Tannin	Present	Present	Present
Carbohydrate	Present	Present	Present
Steroids	Absent	Absent	Absent

Table 2: Results of different extracts during cotton pellet induced granuloma in rats

Group No.	Group	Dose (mg/kg)	Weight of Granulation tissue in mg	% Inhibition of Granulation tissue formation
A	Control	10 ml/kg	37.49 ± 2.54	---
B	Standard (Indomethacin )	2.5	15.84 ± 1.87	57.75
C	Aqueous extract	400	23.70 ± 1.63	36.78
D	Aqueous extract	600	17.12 ± 1.79	54.33
E	Methanolic extract	400	21.94 ± 1.28	41.80
F	Methanolic extract	600	16.93 ± 0.76	54.84
G	Acetone extract	400	25.84 ± 2.26	31.08
H	Acetone extract	600	19.45 ± 1.95	48.12

Results are expressed as Mean ± SEM.  $p < 0.05$  compared to control  $n = 6$

Figure 1: Percentage inhibition of granulation tissue formation in aqueous extract



weight of the cotton pellet is the amount of granulation tissue formed<sup>13, 14, 15</sup>.

The percentage inhibition of granulation tissue formation was measured by the following formula:

$$\% \text{ Inhibition} = (X - Y)/X * 100$$

Where X = mean increase in cotton pellet weight of rats in the control group

& Y = mean increase in cotton pellet weight of rats in the drug treated group

Grouping of animals

Group A - Control (normal saline 10ml/kg b.w.)

Group B - Standard (Indomethacin drug 2.5 mg/kg b.w.)

Group C - Aqueous extract of test drug (400mg/kg b.w.)

Group D - Aqueous extract of test drug (600mg/kg b.w.)

Group E - Methanolic extract of test drug (600mg/kg b.w.)

Group F - Methanolic extract of test drug (600mg/kg b.w.)

Group G - Acetone extract of test drug (400mg/kg b.w.)

Group H - Acetone extract of test drug (600mg/kg b.w.)

### STATISTICAL ANALYSIS

The statistical significance of obtained results was validated by two-way analysis of variance (ANOVA).

The level of significance was fixed between  $p < 0.05$  –  $p < 0.01$ .

### RESULTS

Pharmacognostical study: The macroscopic examination of the powder revealed that its texture is slightly rough, the colour is light brown and it has a sweet odour. It is sour and slightly astringent in taste.

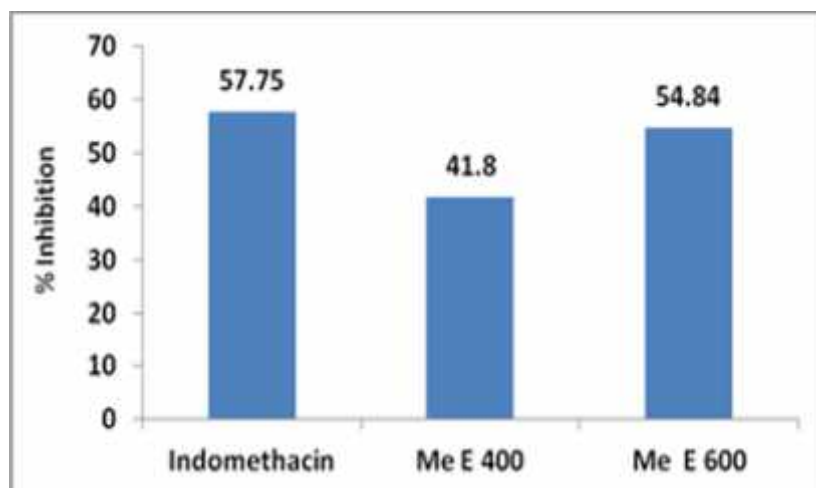
The microscopic characteristics of the powder examined under the compound microscope revealed the presence of crystals and starch grains in large amounts, cork cells, raphides and parenchymatous cells.

Physiochemical analysis: The moisture content was observed to be 8.0% w/w. The total ash content was found to be 5.005% w/w consisting of the acid insoluble ash of 1.67% w/w and water soluble ash content of 3.34% w/w. The yield of dried acetone extract of test drug was 3.75% w/w while that of the aqueous extract was 3.57% and of Methanolic extract was 7.01%. (table 1)

Phytochemical screening: The preliminary phytochemical screening of the various extracts revealed the presence of flavonoids, tannins and carbohydrates as given in table 1.

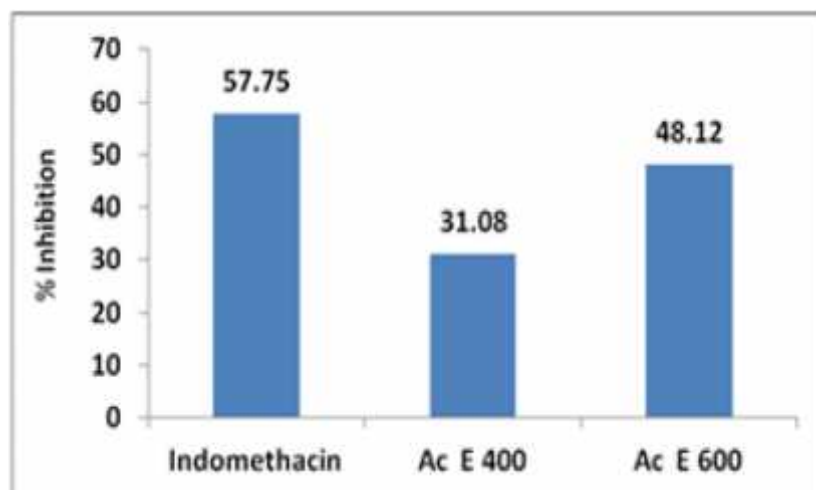
Acute Toxicity: No mortality was found in the acute toxicity studies up to the dose of 1000 mg/kg body weight in the different extracts of the of test drug during the first

Figure 2: Percentage inhibition of granulation tissue formation in methanolic extract



and 31.08 % inhibition respectively during the study

Figure 3: Percentage inhibition of granulation tissue formation in acetone extract



(figures 1-4).

24 hours and even up to the next 14 days. Besides this, no significant changes occurred in the behavioural pattern, motor activity, salivation, sedation, convulsion, etc during this study.

Evaluation of Cotton pellet induced granuloma formation: The results of the formation of granulation tissue around the transplanted sterilised cotton in the groin region of the rat have been shown in table 2. The mean values of pre and post readings of each group were taken and the percentage inhibition of granulation tissue formation calculated with reference to the control group.

Overall,

the standard drug Indomethacin showed more inhibition of granuloma formation (57.75%) than the different doses of various extracts of test drug. The 600 mg/kg dose of methanolic, aqueous and acetone extracts showed 54.84 %, 54.33 % and 48.12 % inhibition respectively when compared with the control group (table 2). Similarly, the 400 mg/kg dose of test drug in case of the methanolic, aqueous and acetone extracts showed 41.80 %, 36.78 %

In terms of overall anti-inflammatory effect, the test drug exhibited highly significant impact of all extract at 600 mg/kg dose which was close to that of the standard drug and a slightly lower efficacy at the dose of 400 mg/kg. Among the three extracts of the test drug evaluated during the study, the methanolic one exhibited the highest inhibition of granulation tissue formation followed by a slightly lower impact in aqueous extract and the lowest effect in case of the Acetone extract.

#### DISCUSSION

Over the past few decades, inflammation has been recognized as a major risk factor for various human diseases. Acute inflammation is short-term, self-limiting and it is easy for host defenses to return the body to homeostasis. The non steroidal anti-inflammatory drugs like indomethacin exhibit anti-inflammatory action due to inhibition in the production of arachidonic acid, prostaglandin and interleukins. Chronic inflammatory responses are predisposed to a pathological progression

Figure 4: Comparative inhibition of granulation in different extracts



of chronic illnesses characterized by infiltration of inflammatory cells, excessive production of cytokines, dysregulation of cellular signaling and loss of barrier function. Therefore, targeting the reduction of chronic inflammation is a beneficial strategy to combat several human diseases<sup>15</sup>.

The test drug was found to result in no toxic symptoms or mortality up to the dose of 1000 mg/kg during the acute toxicity study of all extracts undertaken before starting the anti-inflammatory experiments on rats. The assessment of the chronic inflammation effect of test drug in different doses of 400 mg/kg and 600 mg/kg of each extract showed significant inhibition of granuloma formation after 5<sup>th</sup> day of the test drug administration following the implantation of cotton pellet in the groin when compared with the control group. The percentage inhibition of granuloma tissue formation at the dose of 600 mg/kg of methanolic and aqueous extracts (54.84% and 54.33% respectively) was similar to the impact of the standard drug, indomethacin (57.75%).

The results of the present experimental analysis of the acetone, aqueous and methanolic extracts of the test drug having equal amounts of the powder of fruits of *Emblica officinalis*, stems of *Tinospora cordifolia* and rhizomes of *Cyperus rotundus* following its pharmacognostical & chemical analysis reveal the presence of common flavonoids, tannin & carbohydrates in all extracts. The polyphenolic type of compounds are mostly found in the plant in the form of secondary metabolites such as flavonoids, tannins, gallic acid, ellagic acid, etc. which possess anti-inflammatory, antipyretic, antioxidant, anti-arthritis and cardio-protective properties<sup>16, 17</sup>.

Flavonoids are widely present in the dietary components such as fruits and vegetables and exhibit a broad spectrum of biological activities for human health including an anti-inflammatory property. Numerous studies have proposed that flavonoids act through a variety of mechanisms to prevent and attenuate inflammatory responses and serve as possible cardio-protective, neuro-protective and chemo-preventive agents<sup>16</sup>.

The study indicated the significant anti-inflammatory pharmacological action of the test drug in terms of dose dependant inhibition of granuloma tissue formation when compared to the control group. The evaluated impact was noticed to be similar to that of the standard drug indomethacin probably due to decrease in the synthesis of prostaglandin & cyclooxygenase enzyme which are responsible for such an effect<sup>18</sup>. The observed anti-inflammatory effect of the test drug could be attributed to the presence of high concentrations of phenolic compounds like flavonoids, tannin, gallic acid, etc. which are known to possess such properties. The chemical study for the isolation of biological compound of the test drug is going on in the laboratory.

#### ACKNOWLEDGEMENTS

This research work was supported by grants from the Department of Science and Technology, Government of West Bengal and we are grateful for that. We are thankful to Sohini Majumdar, JRF, Department of Dravyaguna, Institute of Post Graduate Ayurvedic Education and Research, Kolkata for providing necessary help during experimental work.

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