

Research Article

ANTI-INFLAMMATORY ACTIVITY OF TRACHYSPERMUM AMMI

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ABSTRACT

Introduction: Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Compounds that reduce inflammation are called anti-inflammatory agents.

Materials and Methods:

Materials: Plant: *Trachyspermum ammi* was collected from wild and authenticated by botanist, dried in shade, size is reduced by mechanical grinder, powdered material was subject to soxhlet extraction with ethanol and the final extract obtained was dried and used for preclinical studies.

Chemicals: Ethanol, water for injection, Carrageenan, Fehling's A, Fehling's B Solution, benedicts reagent, Barfoeds reagent, bials reagent, aniline acetate, HCL, schwinoff's reagent, tannic acid and drugs (Paracetamol) used for the study were of analytical grade.

Animals: After the IAEC approval twenty-four (24) albino male Sprague dawley rats weighing between 120-150 grams were used for the study. They were kept in polypropylene cages and allowed to acclimatize to the environment for two weeks before the commencement of the experiment. The animals were fed with standard animal pellet grower mash and allowed access to water ad libitum. Experimental Induction of Paw edema in rats Male or female Sprague-Dawley rats with a body weight between 100 and 150 g are used.

Methodology: The animals are starved overnight. To ensure uniform hydration, the rats receive 5ml of water by stomach tube (controls) or the test drug dissolved or suspended in the same volume. Thirty minutes later, the rats are challenged by a subcutaneous injection of 0.05ml of 1% solution of carrageenan into the plantar side of the left hind paw. The paw is marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume is measured by plethysmograph immediately after injection, again 3hrs, 3.5hrs, 4hrs.

Determination of Paw edema: The increase of paw volume after 3hr, 3.5hr, 4hr is calculated as percentage compared with the volume measured immediately after injection of the irritant for each animal. Effectively treated animals show much less edema.

Result: The difference of average values between treated animals and control groups is calculated for each time interval and statistically evaluated. The difference at the various time intervals gives some hints for the duration of the anti-inflammatory effect. In Statistical analysis all the data were expressed as mean $\pm$ SEM. Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Duncan's multiple range tests. The results were considered statistically significant if the P value $\leq$ 0.05.

KEY WORDS

Ajwain, Antiinflammatory activity, *Trachyspermum ammi*, Rat paw edema, Carrageenan.,

INTRODUCTION

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Inflammation is not a synonym for infection, even in cases where inflammation is caused by infection. Although infection is caused by a microorganism, inflammation is one of the responses of the organism to the pathogen. However, inflammation is a stereotyped response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen. (Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardin SE, February 2007).

Progressive destruction of the tissue would compromise the survival of the organism. However, chronic inflammation can also lead to a host of diseases, such as hay fever, Periodontitis, atherosclerosis, rheumatoid arthritis, and even cancer (e.g., gallbladder carcinoma). It is for that reason that inflammation is normally closely regulated by the body. Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the

tissue from the inflammatory process. (Abbas A.B.; Lichtman A.H et al,2009).

Inflammation is mainly caused by the burns, chemical irritants Toxins, Infection, pathogens, Physical injury, blunt or penetrating, Immune reactions due to hypersensitivity, ionizing radiation, foreign bodies, including splinters, dirt and debris, Stress, Trauma and Alcohol. (Cotran; Kumar, Collins Robbins Pathologic Basis of Disease).

Acute inflammation begins within seconds to minutes following the injury of tissues. The damage may be purely physical, or it may involve the activation of an immune response. Three main processes occur:

- Increased blood flow due to dilation of blood vessels (arterioles) supplying the region
- Increased permeability of the capillaries, allowing fluid and blood proteins to move into the interstitial spaces
- Migration of neutrophils (and perhaps a few macrophages) out of the capillaries and venules and into interstitial spaces. If the condition causing acute inflammation is not resolved, the inflammation may pass to a longer-term chronic phase. Also, some pathogens by their nature tend to directly provoke chronic rather than acute inflammation. Many of the features of acute inflammation continue as the inflammation becomes chronic, including increased blood flow and increased capillary permeability. Accumulation of white blood cells also continues, but the composition of the cells changes.

MATERIALS AND METHODS

Materials: Plant: *Trachyspermum ammi* was collected from wild and authenticated by botanist. The plant was chopped and dried in shade. It was subjected to size reduction by mechanical grinder. The powdered material was subjected to soxlet extraction with ethanol; the extract

obtained was dried and used for preclinical studies.

Chemicals: All the chemicals: Ethanol, water for injection, Carragenan, Fehling's A, Fehling's B Solution, benedicts reagent, Barfoeds reagent, bials reagent, aniline acetate, HCL, schwinoff's reagent, tannic acid and drugs (Paracetamol) used for the study were of analytical grade.

Animals: Twenty-four (24) albino male Sprague dawley rats weighing between 120-150 grams were used for the study. They were kept in polypropylene cages and allowed to acclimatize to the environment for two weeks before the commencement of the experiment. The animals were fed with standard animal pellet grower mash and allowed access to water ad libitum.

Methods: Experimental Induction of Paw edema in rats Male or female Sprague-Dawley rats with a body weight between 100 and 150 g are used. The animals are starved overnight. To ensure uniform hydration, the rats receive 5ml of water by stomach tube (controls) or the test drug dissolved or suspended in the same volume. Thirty minutes later, the rats are challenged by a subcutaneous injection of 0.05ml of 1% solution of carrageenan into the plantar side of the left hind paw. The paw is marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume is measured by plethysmograph immediately after injection, again 3hrs, 3.5hrs, 4hrs. (Winter et al).

Determination of Paw edema: The increase of paw volume after 3hr, 3.5hr, 4hr is calculated as percentage compared with the volume measured immediately after injection of the irritant for each animal. Effectively treated animals show much less edema. The difference of average values between treated animals and control groups is calculated for each time interval and statistically evaluated. The difference at the various time intervals gives some hints for the duration of the

anti-inflammatory effect. 4.2.3 Statistical Analysis All the data were expressed as mean $\pm$ SEM. Statistical comparisons were performed by one way analysis of variance (ANOVA) followed by Duncan's multiple range tests (Duncan et al., 1977). The results were considered statistically significant if the P value $\leq$ 0.05.

METHODOLOGY

The plant selection was done based on the through literature survey. The plant procured from the cultivator was send to botanist for authentication and was identified as *Trachyspermum ammi*.

SIZE REDUCTION: The plant was procured from commercially from cultivator. The plant was chopped and dried in shade to ensure that the volatile components are not lost. It was then subjected to size reduction by mechanical grinder. The powdered material was subjected to soxhlet extraction with ethanol; the extract obtained was dried and used for preclinical studies.

Hot Continuous Extraction (Sохhlet): In this method, the finely ground crude drug is placed in a porous bag or "thimble" made of strong filter paper, which is placed in extraction chamber of the Soxhlet apparatus. The extracting solvent in flask is heated, and its vapors condense in condenser. The condensed extractant drips into the thimble containing the crude drug, and extracts it by contact. When the level of liquid in extraction chamber rises to the top of siphon tube, the liquid contents of extraction chamber; siphon into flask. This process is continuous and is carried out until a drop of solvent from the siphon tube does not leave residue when evaporated. The advantage of this method; is that large amounts of drug can be extracted with a much smaller quantity of solvent. This affects tremendous economy in terms of time, energy and consequently financial inputs. At small scale, it is

employed as a batch process only, but it becomes much more economical and viable when converted into a continuous extraction procedure on medium or large scale.

STEPS OF EXTRACTION:

The following steps were sequentially involved in the extraction procedure.

A) Soaking of the powdered plant materials: 50 grams of the powdered drug was weighed accurately & taken in a beaker. It is moistened with a little volume of the solvent such that the entire material is uniformly moistened by the solvent.

B) Packing in the thimble: A thimble was prepared to accommodate the previously wetted powder. The thimble is prepared in such a way that its height is prepared in such a way that its height is about 2/3rd of the height of the body so as to ensure proper evaporation & condensation of the solvent.

C) Addition of solvent: The solvent was added in such a way that the entire thimble is soaked by the first cycle of solvent. Two such cycle of solvent was taken so as to ensure complete soaking of the thimble & its content.

D) Process of extraction: The extraction process was started & is continued till the whole of the solvent become colorless & a semi solid extract is obtained.

SOLVENT EVAPORATION:

Trace amount of the solvent may be present in the semi solid extract obtained. This solvent remnant was removed by subjecting it to desiccation. A

desiccator with activated silica gel was used for this purpose. Percentage yield is calculated.

PHYTOCHEMICAL ANALYSIS:

The phytochemical evaluation of the ethanolic extract of Ajwain is studied for the various phytochemical constituents. (Practical Pharmacognosy Dr.K.R khandelwal).

PRECLINICAL STUDY MODEL

Carragenan-induced rat paw edema method ×100
Male or female Sprague-Dawley rats with a body weight between 100 and 150 g are used. The animals are starved overnight. To ensure uniform hydration, the rats receive 5ml of water by stomach tube (controls) or the test drug dissolved or suspended in the same volume. Thirty minutes later, the rats are challenged by a subcutaneous injection of 0.05ml of 1% solution of carrageenan into the plantar side of the left hind paw. The paw is marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume is measured by plethysmograph immediately after injection, again 3h, 3.5h, 4h after challenge. Rats of Male Sprague-Dawley strain weighing 150–200 g is overnight fasted. The animals are injected subcutaneously with 0.05 ml of 1% solution of carrageenan on the plantar surface of the left hind paw. The paw is marked with ink at the level of lateral malleolus and immersed in the mercury column of a plethysmometer for measuring the paw volume. They are divided into four groups of six each.

The animals are divided into four groups.

- **Group 1: Untreated group** the animals receive no **Inflammation** and no vehicle.



Fig: 1 Healthy animal

- **Group 2: Control group** the animals with **Induced inflammation** and vehicle.



Fig: 2 Inflammation of rat paw edema

- **Group 3: Standard group** the animals with carragenan **Induced inflammation** and **Paracetamol (500 mg/kg)** dissolved in distilled water using an oral gavage.



Fig:3 Administration of Standard drug Paracetamol

- **Group 4: Test group** the animal's carrageenan Induced inflammation and Ethanolic extract of *Trachyspermum ammi*(100mg/kg) extract dissolved in distilled water using an oral gavage.



Fig 4: Administration of Test Drug Extract (*Trachyspermum ammi*)

The animals are carefully observed After 3hr of carrageenan injection. They are placed in individual cages for recovery and should have free access to food and water. The experimental room is maintained between 25°C and 28°C with natural light and dark cycles. The Increase in paw volume at 3h is calculated as percentage compared with the volume measured immediately after the injection of carrageenan for each animal. The difference of average values between treated animals and control group is calculated. Calculate the inflammation and response percentage for each drug.

Steps of standard operating procedure:

Randomization and Grouping: Random assignment are the process of assigning individuals at random to groups or to different groups in an experiment. In the statistical theory of design of experiments, randomization involves randomly allocating the experimental units across the treatment groups. The animals were separated according to weights and they were grouped into standard, test I, and control group following Z route i.e. it contains animals from higher weight to lower weight.

- **Experimental Induction of Paw edema in rats:** Male or female Sprague-Dawley

rats with a body weight between 100 and 150 g are used. The animals are starved overnight. To ensure uniform hydration, the rats receive 5ml of water by stomach tube (controls) or the test drug dissolved or suspended in the same volume. Thirty minutes later, the rats are challenged by a subcutaneous injection of 0.05ml of 1% solution of carrageenan into the plantar side of the left hind paw. The paw is marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume is measured plethysmograph immediately after injection 3h, 3.5h, 4hr.

- **Determination of Paw edema:** Increase of paw volume after 3 or 4 h is calculated as percentage compared with the volume measured immediately after injection of the irritant for each animal. Effectively treated animals show much less edema. The difference of average values between treated animals and control groups is calculated for each time interval and statistically evaluated. The difference at the various time interval give some hints

for the duration of the anti-inflammatory effect.

RESULTS

YIELD: Percentage yield was found to be 92%

PHYTOCHEMICAL EVALUATION

The preliminary phytochemical investigation of extract of *Syzygium cumini* shows presence of carbohydrates, tannins, alkaloids, saponins and flavonoids in ethanolic extract. The presence of

Carbohydrates was confirmed by Molisch's test, Fehling's test & Barfoed's test; while, Alkaloids by Mayer's test, Wagner's test & Dragendroff's test. The presence of Flavonoids was confirmed by Shinoda test & Lead acetate test. While of Glycosides by Borntrager's test; Phenolics & Tannins by Bromine water test; Saponins by Foam test. As, ethanolic extract shows presence of most of these compounds, its extract was selected for this study (Table 1).

Table 1: Phytochemical Investigation of Trachyspermum Ammi

Sr. No.	Name Of Compounds	Ethanolic Extract Of Trachyspermum Ammi
1.	Alkaloids	+++
2.	Carbohydrates	+++
3.	Glycosides	+
4.	Saponins	+
5.	Fats and Oils	+
6.	Flavonoids	+++
7.	Tannins	+++
8.	Steroids	+
9.	Proteins	-

+ = slightly present; ++ = moderately present; +++ = highly present; - = Absent

Table 2: Standard Drug Inhibition of Rat Paw Edema

S.NO	Body Weight (gm)	0 HR	3HRS	3.5 HRS	4 HRS
1	97.4	0.5	0.7	0.6	0.5
2	99.8	0.4	0.9	0.8	0.5
3	114	0.5	0.7	0.7	0.5
4	101.2	0.5	0.7	0.5	0.4
5	102.4	0.5	0.8	0.5	0.4
6	94.2	0.4	0.5	0.5	0.4

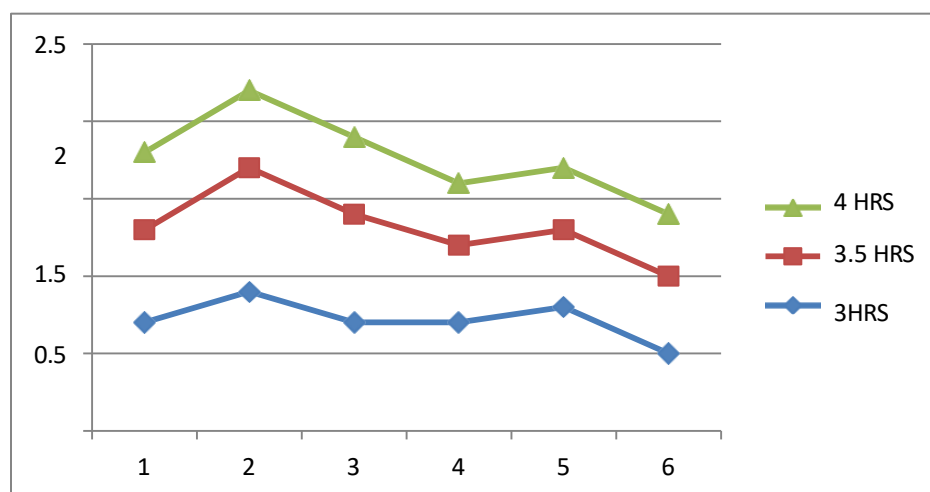


Fig: 5 The graph showing the line diagram of plethysmograph reading of the standard drug paracetamol at various time intervals.

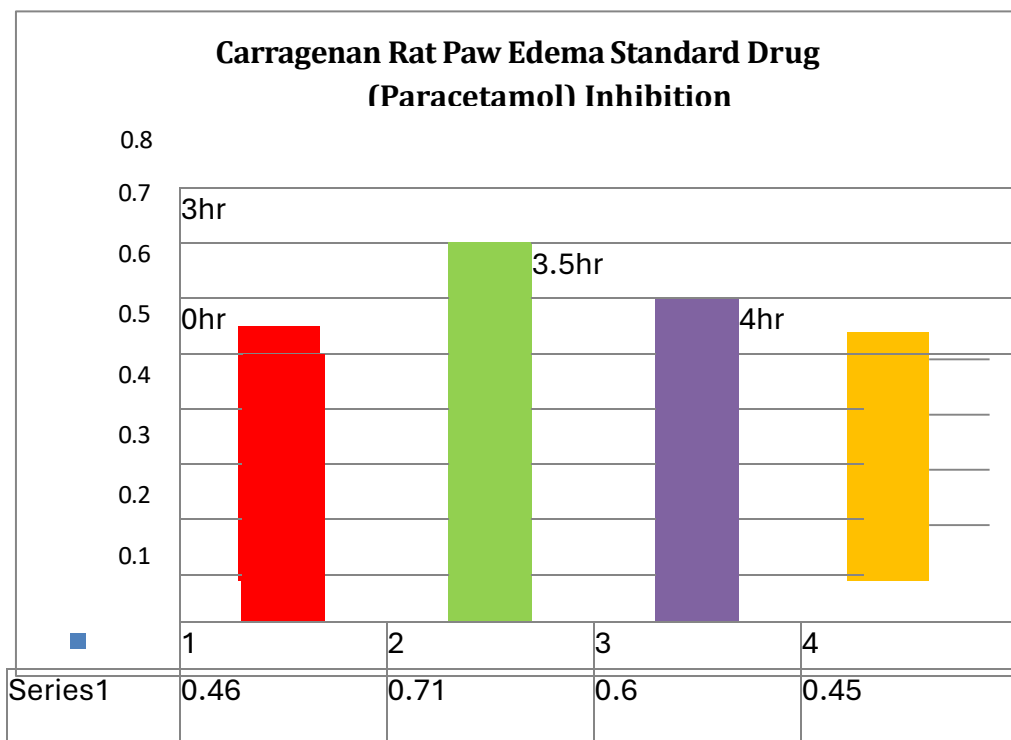


Fig: 6 The graph showing the Bar diagram of plethysmograph reading of the standard drug paracetamol at various time intervals.

Table 3: Test Drug Inhibition of Rat Paw Edema

S.NO	Body Weight(gm)	0 HR	3HRS	.5 HRS	4 HRS
1	94	0.4	0.5	0.5	0.4
2	92.2	0.4	0.8	0.7	0.4
3	98.4	0.5	0.6	0.6	0.5
4	90.6	0.5	0.7	0.5	0.5
5	83.8	0.5	0.8	0.5	0.5
6	113.8	0.4	0.7	0.6	0.4

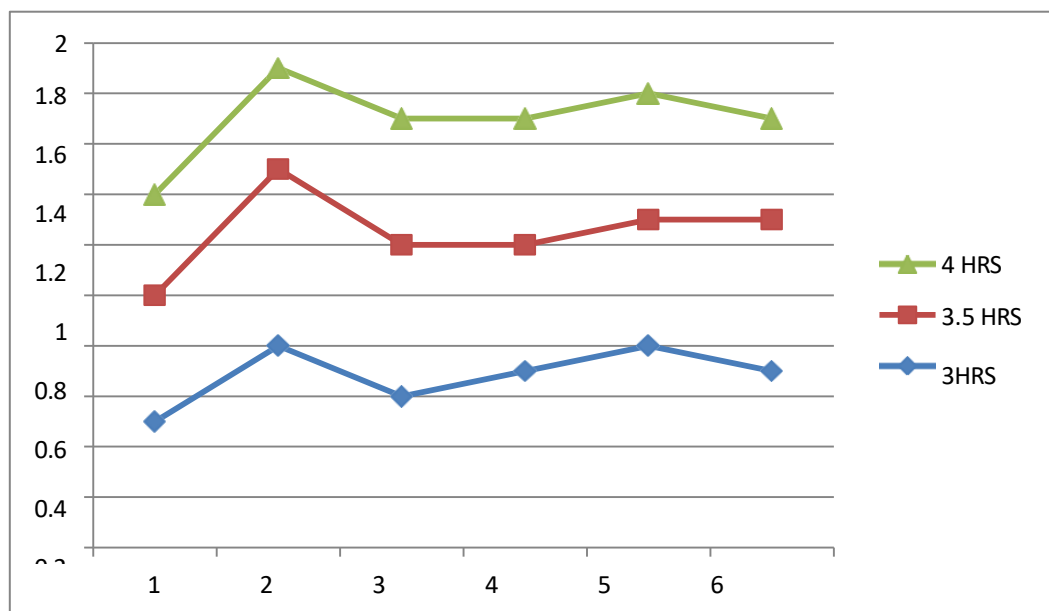


Fig: 7 The graph showing the line diagram of plethysmograph reading of the test drug at various time intervals.

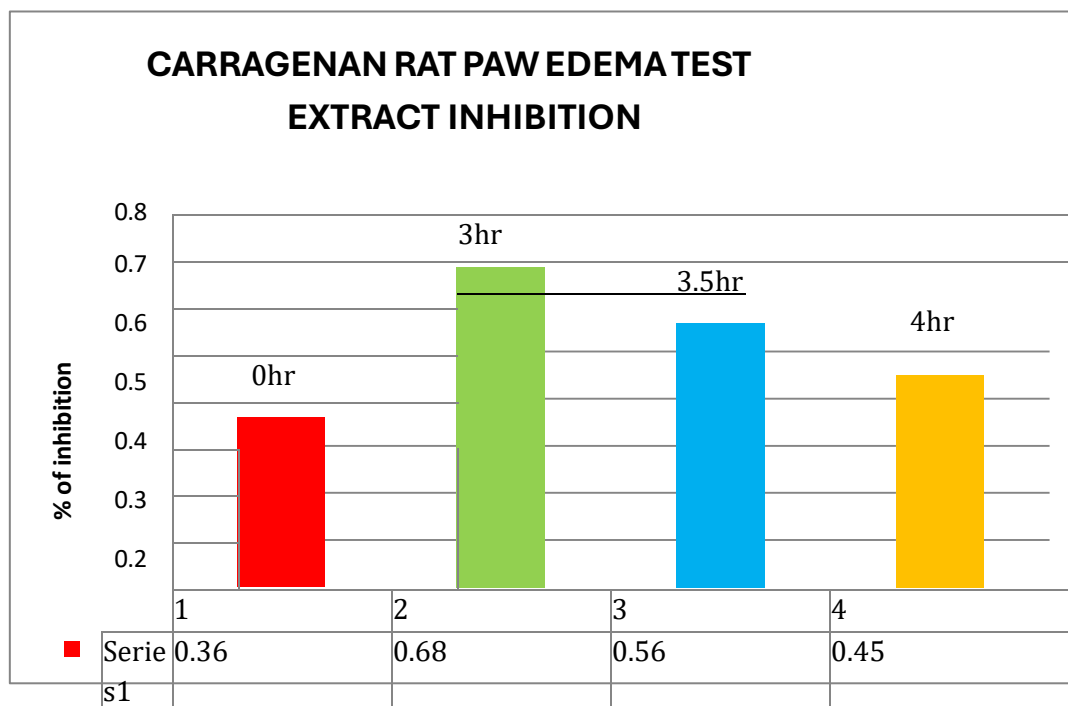


Fig: 8 The graph showing the Bar diagram of plethysmograph reading of the test drug at various time intervals.

TABLE 4: Anti-inflammatory activity of ethanolic extract of *Trachyspermum ammi* on carrageenan induced paw edema model.

Groups	Dose (mg/kg)	Paw volume (Mean±S.E)	Paw edema in ml (Mean ±S. E)		
			3hr	3.5hr	4hr
Control	0.5%	0.5±0.05	0.43±0.06	0.63±0.04	0.7±0.03
Paracetamol	500mg/kg	0.55±0.01	0.07±0.04	0.60±0.01	0.41±0.05
Ethanolic extract of test drug	100mg/kg	0.45±0.05	0.68±0.01	0.56±0.05	0.45±0.04

Significant at $p < 0.05$ values calculated by ANOVA followed by control group.

DISCUSSION:

The present study the animal was divided into three groups Standard group, test group, and Untreated group. The untreated group constantly shows the inflammation of carrageenan induced of left hind paw edema. Plethysmography used for these experimental studies. so, the compounds screen through this method and show the effect of drug which have potential to treat inflammation.

The results from the present study suggest that the extract of *Trachyspermum ammi* exhibited

significant Anti-inflammatory effect. Inflammation has different phases the first phase is caused by an increase in vascular permeability the second one by infiltrate of leukocytes and third one by granuloma formulation, by activating the cyclo-oxygenase, the levels of prostaglandin, especially PGE₂, increase markedly and its production provokes inflammation and pain. Therefore, we assume that some active metabolites of the extract in this study could inhibit cyclo-oxygenase activity. (Coussens LM, Werb Z. et al).

The most widely used primary test to screen anti-inflammatory agents is to measure the ability of a compound to reduce local oedema induced in rat paw following the injection of irritants such as carragenan. (Chopra RN et al). It is also suitable for assessing of the natural products and is believed to be biphasic. The first phase of 1hr involves the release of serotonin and histamine while the second of phase of the next 1hour is mediated by prostaglandin especially PGE₂. The significant reduction as well as inhibitory effect of the extract on the Carragenan-induced oedema paw volume is an indication of the anti-inflammatory potentials of the plant.

Therefore, the result of this study supports the use of the plant extract to suppression of paw oedema in the last phase could probably be due to inhibition in the release of early mediators such as Histamine, Serotonins & Kinins as well as Cyclo-oxygenase. The phytochemical screening of the extracts of Trachyspermum ammi is the presence of Carbohydrates, Flavonoids, Glycosides, Terpenoids Saponins, and Tannins. Flavonoids are capable to inhibit the Prostaglandin synthetase, more specifically the endoperoxidase, and reported to produce anti-inflammatory effect. The Presence of flavonoids and other Phytochemicals may be contributing to the anti-inflammatory potential. (Joy PP et al). The results provide a scientific basis for the utilization of this herb in traditional medicine for the treatment of inflammatory diseases. (Samuel Mathew et al).

CONCLUSION

The aim of this study is to determine the effects of Trachyspermum ammi extract on some physiological profiles in Carragenan induced Sprague dawley rats. The research work demonstrates that Trachyspermum ammi resulted in a significant decrease on rat paw edema. The study also indicated the presence of antioxidant principles in the extract of trachyspermum ammi thus also supports the

mechanism of action of carragenan. The result of the trachyspermum ammi extract shown was more significant than standard drug. Further studies need to be done to characterize the active components of the ethanolic extract of Trachyspermum ammi and its mechanism of action. The plant contains Thymine, Thymol, Carvone, Saponin, Flavonoids, Tannins, Alkaloids, Glycosides, Steroids and Terpenoid as Phytochemical compounds present which may also be responsible for the antioxidant and anti-inflammatory activity.

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