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EVALUATION OF ANTI INFLAMMATORY ACTIVITY OF VARIOUS EXTRACTS OF CARROT AGAINST CARRAGEENAN INDUCED IN RAT MODEL

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ABSTRACT

Daucus carota is a biennial plant that grows a rosette of leaves in the spring and summer, while building up the stout taproot that stores large amounts of sugars for the plant to flower in the second year. Soon after germination, carrot seedlings show a distinct demarcation between the taproot and the stem. The latter is thicker and lacks lateral roots. At the upper end of the stem is the seed leaf. The first true leaf appears about 10–15 days after germination. Subsequent leaves, produced from the stem nodes, are alternating (with a single leaf attached to a node, and the leaves growing in alternate directions) and compound, and arranged in a spiral. The leaf blades are pinnate. Taproots typically have a long conical shape, although cylindrical and round cultivars are available. The root diameter can range from 1 cm (0.4 in) to as much as 10 cm (4 in) at the widest part. The root length ranges from 5 to 50 cm (2.0 to 19.7 in), although most are between 10 and 25 cm (4 and 10 in). The main objective of the present research work was to isolate the bioactive molecules and evaluate the *in vivo* anti inflammatory activity of ethanolic, methanolic and chloroform extracts of carrot (EEC, MEC and CEC) of ***Daucus carota subsp. Sativus.*** The Anti-inflammatory activity was performed by Carrageenan induced paw edema method in rats. The present experimental data displayed that all the extracts (EEC and MEC and CEC) of *Daucus carota subsp. Sativus* very good anti inflammatory activity at 200 mg/kg body weight. All the extracts exhibited highest anti inflammatory activity at 120 min. When compared with standard drug diclofinac sodium (10 mg/kg i.p) it was found that CEC exhibited good anti-inflammatory activity and the percentage protection of EEC and MEC and CEC and standard drug diclofinac sodium for anti inflammatory activity were found to be, 44.95%, 39.18%, 48.64%, 70.27% etc.

KEYWORDS: Germination, pinnate, bioactive molecules, anti inflammatory activity, Carrageenan etc.

INTRODUCTION

Inflammation (Latin, inflammatio) is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, damaged cells, or irritants.[1] and is a protective response involving immune cells, blood vessels, and molecular mediators. The function of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and to initiate tissue repair. The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function. Inflammation is a generic response, and therefore it is considered as a mechanism of innate immunity, as compared to adaptive immunity, which is specific for each pathogen.[2] Too little inflammation could lead to progressive tissue destruction by the harmful stimulus (e.g. bacteria) and compromise the survival of the organism. In contrast, chronic inflammation may lead to a host of diseases, such as hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and even cancer (e.g., gallbladder carcinoma). Inflammation is therefore 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 series 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, such as mononuclear cells, and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.[3] Inflammation is not a synonym for infection. Infection describes the interaction between the action of microbial invasion and the reaction of the body's inflammatory defensive response the two components are considered together when discussing an infection, and the word is used to imply a microbial invasive cause for the observed inflammatory reaction. Inflammation on the other hand describes purely the body's immunovascular response, whatever the cause may be. But because of how often the two are correlated, words ending in the suffix -itis (which refers to inflammation) are sometimes informally described as referring to infection. For example, the word urethritis strictly means only "urethral inflammation", but clinical health care providers usually discuss urethritis as a urethral infection because urethral microbial invasion is the most common cause of urethritis. It is useful to differentiate inflammation and infection as there are many pathological situations where inflammation is not driven by microbial invasion for example, atherosclerosis, type III hypersensitivity, trauma, ischaemia. There are also pathological situations where microbial invasion does not result in classic inflammatory response for example, parasitosis, eosinophilia.[4]

Acute inflammation is a short-term process, usually appearing within a few minutes or hours and begins to cease upon the removal of the injurious stimulus.[5] It involves a coordinated and systemic mobilisation response locally of various immune, endocrine and neurological mediators of acute inflammation. In a normal healthy response, it becomes activated, clears the pathogen and begins a repair process and then ceases.[6] It is characterized by five cardinal signs.[7] An acronym that may be used to remember the key symptoms is "PRISH", for pain, redness, immobility (loss of function), swelling and heat. The traditional names for signs of inflammation come from Latin: dolor (pain), calor (heat). rubor (redness), tumor (swelling) and functio laesa (loss of function).[8] The first four (classical signs) were described by Celsus (ca. 30 BC–38 AD),[9] while loss of function was added later by Galen,[10] even though the attribution is disputed and the origination of the fifth sign has also been ascribed to Thomas Sydenham.[11] and Virchow.[5,7] Redness and heat are due to increased blood flow at body core temperature to the inflamed site; swelling is caused by accumulation of fluid; pain is due to the release of chemicals such as bradykinin and histamine that stimulate nerve endings. Loss of function has multiple causes.[7] Acute inflammation of the lung (pneumonia) does not cause pain unless the inflammation involves the parietal pleura, which does have pain-sensitive nerve endings.[7]

Inflammation is a process that is accompanied by the local liberation of chemical mediators like histamine, 5-hydroxy tryptamine, bradykinin and ecosanoids. Ecosanoids are extremely potent compounds involved in most types of inflammation and are formed in almost tissue in the body Thus, inhibition of their biosynthesis is the mainstay of anti-inflammatory therapy for example corticosteroids inhibit the formation of arachidonic acid (AA), the starting compound of ecosanoids. Normally AA is further metabolized by the enzyme cyclooxygenase (COX) to a group of compounds called prostaglandins (PG_S). It has been shown that PGE_2 and PGI_2 cause erythema and increase local blood flow and PGE_2 and $PG_{2\alpha}$ cause intense local pain when given i. m, s. c and PGE_1 causes itching and finally PGE_2 is associated with production of fever. Inhibition of COX by NSAIDS prevents formation of PG_S and brings about its therapeutic actions. However, two things must be kept in mind while dealing with NSAIDS: a) In general , these drugs only offer symptomatic relief without affecting the underlying disease and appropriate drugs need to be given simultaneously to correct the pathological state e.g appropriate antibiotic in a febrile patent and (b) by virtue of its COX inhibiting property the use of these drugs also leads to the manifestation of certain common adverse effects, which may actually limit the use of an NSAID e.g aspirin and gastric ulceration.

In the prostaglandin (PG) synthetic path way, cyclooxygenase (COX) converts AA to unstable intermediate PGG_2 and PGH_2. It is suggested that there are two forms of Cyclooxygenase, called as COX-1 and COX-2. COX-1 is constitutive and found in blood vessels, stomach, and kidney, while COX-2 is induced in settings of inflammation by cytokines and inflammatory mediators. Aspirin covalently binds with both COX-1 and COX-2, resulting in irreversible inhibition of Cyclooxygenase activity. Aspirin by binding to COX-1 prevents the binding of AA to the active site of the enzyme and thus, inhibit PG synthesis Althuogh modification of COX-2 by aspirin also inhibits COX activity, but this isoform now synthesizes 15-HETE. The majority of the NSAIDS, in contrast to aspirin serve as reversible competitive inhibitors of COX activity. Most of the presently available NSAIDS non selectively inhibit the COX -1 and COX-2, isoforms or have limited selectivity for the constitutive COX-1 isoform. However, nabumetone, a new NSAID, preferentially inhibits COX-2 .This agent has anti-inflammatory activity with little ulcerogenic potentials. Similarly new drugs like nimesulide and meloxicam are also reported to have greater affinity for COX-2 as compared to COX-1. Thus

the effort now is to develop $NSAID_S$ which have greater affinity for COX-2 than COX-1.[12]

Experimental Phytochemistry

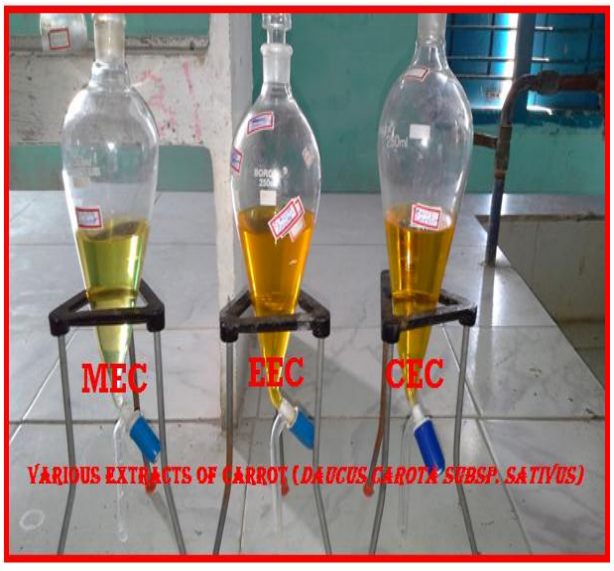
Materials and method

Drugs and chemicals: The standard drug and diclofenac sodium (Anti-inflammatory) was purchased from Local Retail Pharmacy Shop and solvents and other chemicals used for the extraction and phytochemical screening used from Institutional Store and were of AR grade**.**

Apparatus: round bottom flask, water condenser, heating mantle, motor and pestle.

Chemicals required: (a) ethanol (b) ethylacetoacetate (c) dichloromethane (d) sodium chloride solution (e) magnesium sulphate.

Methodology:[13] Weigh 20 g of carrot paste (root can be mashed or grinded to prepare a paste) into a 250 ml round-bottomed flask. Add 50 ml of ethanol and 60 ml of dichloromethane. Heat the mixture under reflux for 5 min on stem-bath with frequent shaking. Filter the mixture under suction and transfer the filtrate to a separating funnel. Wash this mixture containing bioactive compounds with three portions of 150 ml each with sodium chloride solution. Dry the organic layer over anhydrous magnesium sulfate. Filter and evaporate most of the solvent in vacuum without heating and obtained ethanolic extract of carrot **(EEC) of *Daucus carota subsp. sativus*.** Same procedure was followed for the preparation of methanolic and chloroform extracts **(MEC, CEC)** of ***Daucus carota subsp. sativus.***



Phytochemical screening: Preliminary Phytochemical screening of **EEC, MEC and CEC** had shown the presence of various bioactive compounds such as carbohydrates, amino acids and peptides, phytosterols, carotenoids, and polyphenols etc.[14-16]

Experimental Pharmacology

Experimental animals: White male albino Wister rats weighing about 200-250 g were used. They were obtained from the animal house of C.L. Baid Metha College of Pharmacy, Chennai. They were kept under observation for about 7 days before the onset of the experiment to exclude any intercurrent infection, had free access to normal diet and water. The animals were housed in plastic well aerated cages at normal atmospheric temperature (25±5 °C) and normal 12- hour light/dark cycle under hygienic conditions. The experimental protocol was approved by Institutional Animal Ethics Committee **(IAEC) of CPCSEA: IAEC/XXIX/12/2015**.

Protocol for the study of acute oral toxicity of EEC, MEC and CEC: In the present study the acute oral toxicity of the each extracts of carrot was performed by acute toxic class method. In this method the toxicity of the extract was planned to test using step wise procedure, each step using three Wister rats. The rats were fasted prior to dosing (food but not water should be withheld) for three to four hrs. Following the period of fasting the animals were weighed and the extract was administered orally at a dose of 2000 mg/Kg b.w. Animals were observed individually after dosing at least once during the first 30 min; periodically the surveillance was carried out for the first 24 hrs with special attention given during the first 4 hrs and daily thereafter, for a total of 14 days.[17]

Evaluation of Anti-inflammatory activity.[18]

Method: Carrageenan Induced Paw Edema Method in Rats

Requirements: Animal Rats (150-200g).

Drugs: Carrageenan (Prepare 1% w/v solution and inject 0.1 ml underneath the planter region). Diclofenac sodium 10 mg/kg, i.p, prepared a stock solution containing 4 mg/ml of the drug and inject 0.5ml/100 g of the body weight of the animal.

Equipment (Plethysmograph)

It is a simple apparatus containing mercury. The mercury displacement due to dipping of the paw can be directly read from scale attached to the mercury column or adjusting the mercury level in arm B to the original A level by moving arm B up/down and noting the volume required to bring the level in both the arms equal.

Procedure

1. Weigh the animal and number them.
2. Make a mark on both the hind paws (right and left) just beyond tibio-tarsal junction, so that every time the paw is dipped in mercury column up to the fixed mark to ensure constant paw volume.
3. Note the initial paw volume (both right and left) of each rat by mercury displacement method.

4. Divide the animals in to three groups each comprising of at least four rats. To one group inject saline and to the second group inject diclofenac sodium and third group inject drug (sample) intra peritoneal route.
5. After 30 min inject 0.1 ml of 1% (w/v) carrageenan in the planter region of the left paw of control as well as diclofenac sodium and drug treated group. The right paw will serve as reference non-inflamed paw for comparison.
6. Note the paw volume of legs of control, diclofenac sodium and extract (sample) treated rats 30, 60, 120 and 180 min after carrageenan challenge.
7. Calculate the % difference in the right and left paw volumes of each animal of control, diclofenac sodium and drug- treated group. Compare the mean % change in paw volume in control, diclofenac sodium and extract (sample) treated animals and express as percent edema inhibition by extracts. The percentage inhibition of paw oedema was calculated by using the following formula.

Percentage protection = [(control-test)/control] ×100.

RESULTS AND DISCUSSION

Acute toxicity studies

a) Acute oral toxicity studies were performed according to the OECD guideline 423 method.
b) This method has been designed to evaluate the substance at the fixed doses and provide information both for hazard assessment and substance to be ranked for hazard classification purposes.
c) The each extract was administered initially at a dose of 2000 mg/kg b.w and 1% CMC (p.o) and observed 14 days mortality due to acute toxicity.
d) Careful observation were made at least thrice a day for the effect on CNS, ANS, motor activity, salivation and other general signs of toxicity were also observed and recorded.
e) Since no sign of toxicity observed at 2000 mg/kg b.w. to the group of animals, the LD_{50} value of the each extract expected to exceed 2000 mg/kg b. w. and represented as class 5 (2000 mg/kg < LD_{50} < 2500 mg/kg).
f) From the toxicity studies the data revealed that all the extracts proved to be non toxic at tested dose levels and well tolerated by the experimental animals as there LD_{50} cut of values > 2000 mg/kg b. w.

Table 1: for the dose selection by acute toxicity class method (OECD) guide lines 423 of EEC.

Sl. No.	Treatment groups	Dose mg/kg	Sign of toxicity	Onset of toxicity	Duration
1.	EEC	200	No	No	14 days
2.	MEC	200	No	No	14 days
3.	CEC	200	No	No	14 days

Evaluation of anti-inflammatory activity: Statistically significant differences with respect to control was evaluated by (ANOVA), Dunnet's test * P<0.05, **P<0.01, NS (Non significant), % (Percentage reduction of edema). Anti-inflammatory activity of the each extracts was evaluated by carrageenan induced paw edema method. The activity was studied at 200 mg/kg b.w. p.o. And then their responses were measured at 30, 60, 120 and 180 min. From the experimental data shown in table 2 displayed that the three different extracts (EEC, MEC and CEC) possessed mild to good anti-inflammatory activity. All the extracts exhibited highest activity at 120 min. When compared with standard drug diclofinac sodium (10 mg/kg i. p) it was found that CEC exhibited good anti-inflammatory activity.

Table 2: for anti-inflammatory activity of various extracts of carrot.

Groups	30 min		60 min		120 min		180 min	
	MEAN ±SEM	%	MEAN ±SEM	%	MEAN ±SEM	%	MEAN ±SEM	%
Control	0.70 ±0.08	-	0.72±0.07	-	0.74±0.34	-	0.72±0.04	-
DF Na. (10mg/kg p.o.)	0.42 ± 0.06**	40.00	0.30±0.05**	58.33	0.22±0.23**	70.27	0.35±0.23**	51.38
EEC (200mg/kg p.o.)	0.53 ±0.08	24.28	0.50±0.04	30.55	0.41±0.03	44.95	0.40±0.14	44.44
MEC (200mg/kg p.o.)	0.57± 0.04*	18.57	0.46±0.12*	36.11	0.45±0.05*	39.18	0.41±0.32*	43.05
CEC (200mg/kg p.o.)	0.54± 0.05**	22.85	0.48±0.06**	33.33	0.38±0.07**	48.64	0.42±0.34**	41.66

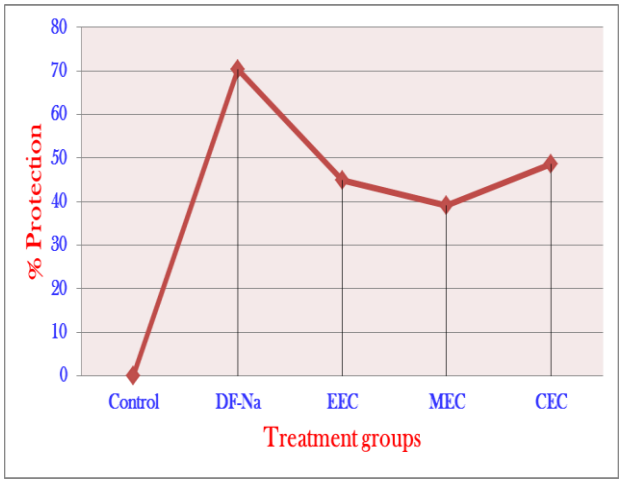


Fig 1: Graphical representation of % protection of various extracts of carrot.

CONCLUSION

Here we reported that the anti-inflammatory activity of prepared various extracts of carrot was evaluated by Carrageenan induced paw oedema method in Wister rats. All the extracts EEC, MEC and CEC exhibited moderate to good anti-inflammatory activity.

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