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Study Of Anti-Inflammatory Activity of *Emblica Officinalis* Fruit in Mice Model

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ABSTRACT

Emblica officinalis, commonly known as amla in Ayurveda, is unarguably the most important medicinal plant for prevention and treatment of various ailments. The present study investigated the anti-inflammatory activity of fruit extract of *Emblica officinalis*, Inflammation in Mices was induced by the subcutaneous injection of formalin is test drug. The effects were comparable to that of the standard anti-inflammatory drugs. The doses are at low concentration is 5mg/kg body weight and at high concentration 10mg/kg. The reduction of inflammation when test drug is given is compared with the standard dug. this review describes a brief overview of the research of anti-inflammatory activity of emblica officinalis in mice models.

KEY WORDS:

Emblica officinalis, Inflammation, mice.

INTRODUCTION

Inflammation is the local response of living mammalian tissues to injury from any agent which could be microbial, immunological, physical or chemical agents. Inflammation is different from infection. Infection is caused by exogenous pathogens while inflammation is the response of the organism to the pathogen or irritating stimuli.

TYPES OF INFLAMATION:

It is of two types: Acute inflammation and Chronic inflammation.

Causes:

The factors that can stimulate inflammation include microorganisms, physical agents, chemicals, inappropriate immunological responses, and tissue death. Infectious agents such as viruses and bacteria are some of the most common stimuli of inflammation. Viruses give rise to inflammation by entering and destroying cells of the body; bacteria release substances called endotoxins that can initiate inflammation. Physical trauma, burns, radiation injury,

and frostbite can damage tissues and also bring about inflammation, as can corrosive chemicals such as acids, alkalis, and oxidizing agents. As mentioned above, malfunctioning immunological responses can incite an inappropriate and damaging inflammatory response. Inflammation can also result when tissues die from a lack of oxygen or nutrients, a situation that often is caused by loss of blood flow to the area.

Signs:

The four cardinal signs of inflammation redness, heat, swelling, and pain were described in the 1st century AD by the Roman medical writer Aulus Cornelius Celsus. Redness is caused by the dilation of small blood vessels in the area of injury. Heat results from increased blood flow through the area and is experienced only in peripheral parts of the body such as the skin. Fever is brought about by chemical mediators of inflammation and contributes to the rise in temperature at the injury. Swelling, called edema, is caused primarily by the accumulation of fluid outside the blood vessels. The pain associated with inflammation results in part from the distortion of tissues caused by edema, and it also is induced by certain chemical mediators of inflammation, such as bradykinin, serotonin, and prostaglandins.

A fifth consequence of inflammation is the loss of function of the inflamed area, a feature noted by German pathologist Rudolf Virchow in the 19th century. Loss of function may result from pain that inhibits mobility or from severe swelling that prevents movement in the area.

ACUTE INFLAMMATION:

It usually occurs for a short (yet often severe) duration. It often resolves in two weeks or less. Symptoms appear quickly. This type restores your body to its state before injury or illness. The process of acute inflammation is initiated by resident immune cells already present in the involved tissue, mainly resident macrophages, dendritic cells, histiocytes, Kupffer cells and mast cells. These cells possess surface receptors known as pattern recognition receptors (PRRs), which recognize two subclasses of molecules: pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). PAMPs are compounds that are associated with various pathogens, but which are distinguishable from host molecules. DAMPs are compounds that are associated with host-related injury and cell damage.

At the onset of an infection, burn or other injuries, these cells undergo activation (one of the PRRs recognizes a PAMP or DAMP) and release inflammatory mediators responsible for the clinical signs of inflammation. Vasodilation and its resulting increased blood flow cause the redness (rubor) and increased heat (calor). Increased permeability of the blood vessels results in an exudation (leakage) of plasma proteins and fluid into the tissue (edema), which manifests itself as swelling (tumour). Some of the released mediators such as bradykinin increase the sensitivity to pain (hyperalgesia, dolor). The mediator molecules also alter the blood vessels to permit the migration of leukocytes, mainly neutrophils and macrophages, to flow out of the blood vessels (extravasation) and into the tissue. The neutrophils migrate along a chemotactic gradient created by the local cells to reach the site of injury. The loss of function (functio laesa) is probably the result of a neurological reflex in response to pain. In addition to cell-derived mediators, several acellular biochemical cascade systems consisting of preformed plasma proteins act in parallel to initiate and propagate the inflammatory response. These include the complement system activated by bacteria and the coagulation and fibrinolysis systems activated by necrosis (e.g., burn, trauma).

Acute inflammation may be regarded as the first line of defense against injury. Acute inflammatory response requires constant stimulation to be sustained. Inflammatory mediators are short-lived and are quickly degraded in the tissue. Hence, acute inflammation begins to cease once the stimulus has been removed.

It can be divided into following two events: Vascular events and Cellular events.

VASCULAR EVENTS:

Alteration in the microvasculature (arterioles, capillaries and venules) is the earliest response to tissue injury. These alterations include hemodynamic and vascular permeability changes. When tissue is first injured, the small blood vessels in the damaged area constrict momentarily, a process called vasoconstriction. Following this transient event, which is believed to be of little importance to the inflammatory response, the blood vessels dilate (vasodilation), increasing blood flow into the area. Vasodilation may last from 15 minutes to several hours.

CELLULAR EVENTS:

Cellular events in inflammation play a crucial role in our body's response to injury and infection. The most important feature of inflammation is the accumulation of white blood cells at the site of injury. Most of these cells are phagocytes, certain "cell-eating" leukocytes that ingest bacteria and other foreign particles and clean up cellular debris caused by the injury. The main phagocytes involved in acute inflammation are the neutrophils, a type of white blood cell that contains granules of cell destroying enzymes and proteins. When tissue damage is slight, an adequate supply of these cells can be obtained from those already circulating in the blood. But, when damage is extensive, stores of neutrophils some in immature form are released from the bone marrow, where they are generated.

CHRONIC INFLAMMATION

Chronic inflammation is inflammation that lasts for months or years. Macrophages, lymphocytes, and plasma cells predominate in chronic inflammation.in contrast to the neutrophils that predominate in acute inflammation. Diabetes, cardiovascular disease, allergies, and chronic obstructive pulmonary disease (COPD) are examples of diseases mediated by chronic inflammation. Obesity, smoking, stress and insufficient diet are some of the factors that promote chronic inflammation. A 2014 study reported that 60% of Americans had at least one chronic inflammatory condition, and 42% had more than one.

Cardinal signs

Common signs and symptoms that develop during chronic inflammation are:

- Body pain.
- Chronic fatigue and insomnia.
- Depression, anxiety and mood disorders.
- Gastrointestinal complications such as constipation, diarrhoea, and acid reflux.
- Weight gain or loss.
- Frequent infections.

MATERIALS AND METHODS

The following drugs and chemicals and other chemicals were used in the present study.
Nemusilide hydrochloride tablets 250 mg
Brand name: ORTHOBID
Manufacturer: AHPL
Emblica officinalis (Amla) extract: Dr. KNV Rao, Nalanda College of Pharmacy, Nalgonda, Telangana.
Normal saline:Brand name: PROLINE
Manufacturer: JOIN HUB PHARMA
Plethysmograph: MKM ENTERPRISES

Experimental Animals

3-months old mices are divided into 4 groups for each method. Each group consist of four animals weighing 25-40 grams were use for the experiment. Permission has been obtained from the Institution Animal Ethical Committee (NCOP/IACE/Approved/70) for animal experiments. Animals were procured from National Institution of Nutrition, Hyderabad. And the experiment had done according to the CPCSEA (Committee for The Purpose of Control and Supervision on Experimental Animals) guidelines. After randomization into various groups, the rats were acclimatized for a period of 2 weeks in the environment before the initiation of the experiment. Their cages were cleaned of waste daily.
They were housed in standard environment condition and fed with standard diet with water and ad libitum. The animal was kept in the cages under the standard conditions (Temperature 25±2°c, 12h dark and 12h light cycle) in animal house in Department of Pharmacology, Nalanda college of Pharmacy, Nalgonda, Telangana.

Plant Collection and Authentication:
Emblica Officinalis plant material was collected in the month of November to December from Nalgonda district, Telangana, India. The plant was identified and authenticated by professor Dr. K N V Rao, principal, Nalanda college of pharmacy, Charlapally, Nalgonda, Telangana. The plant specimen was submitted in department of pharmacognosy, Nalanda College of Pharmacy, Nalgonda.

Preparation of Ethanolic Extract:
The plant fruits were collected and washed thoroughly. Then fruits were separated from the plant and the fruits were cut into small pieces by omitting the seeds in it and shade dried for 10 days. After drying the fruits were coarsely powdered. Then it was kept for maceration process by using methyl alcohol as solvent

Induction of drugs:
Inflammation was induced by using 0.1 ml of formalin.

Experimental Design:
Animals are divided into 4 groups for each group consist of 4 animals.

Formalin induced paw oedema method:
GROUP - I (CONTROL):
Animals receives only Formalin.
GROUP - II (NORMAL SALINE):
Inflammation induced by injecting 0.1 ml of 1% formalin in a single dose into the sub plantar region of right-hand paw.
GROUP - III (STANDARD DRUG):
0.1ml of 1% formalin induced inflammatory micereceived standard Nimesulide in a dose of 10mg/kg body weight by sub cutaneous route 30 minutes prior.
GROUP – IV (PLANT EXTRACT 200mg/kg):
0.1ml of 1% formalin induced inflammatory mice received ethanolic extract in a dose of 200 mg/kg body weight by sub cutaneous route 30-60 minutes prior.

Acute inflammation was induced by sub cutaneous route of 0.1ml of 1% freshly prepared suspension of formalin into the right hind paw of each mice. The paw volume was measured at 30mins,1hr,2hr and 3hr after injection of formalin using paleothermometer. Methanolic extract at varying doses based on the design of the experiment was administered for each group and except control group. ethanolic extract

is in sub cutaneous route and formalin is injected to paw 30mins prior to the injection of imesulide. The volume is measured just before and 30mins, 1hr, 2hr and 3hr after administration of formalin by the volume displacement method using paleothermometer.

RESULT:

CONTROL GROUP

Sl.no	Animal wight	Paw Volume (before inflammation)	Inflammatory agent (0.1%formalin)	Paw volume			
				15min	30min	45min	1hr
1	20-30gm	70	0.1	68	65	63	62
2	25-35gm	80	1.0	72	74	70	72
3	30-35gm	82	1.5	52	50	54	56
4	35-40gm	70	2.0	68	66	66	65

NORMAL SALINE

Sl.no	Animal Weight	Paw Volume	Normal Saline	Inflammatory agent	Paw volume			
					15min	30min	45min	1hr
1	20-30gm	70	0.5	0.9	70	65	60	59
2	25-35gm	72	1.2	0.95	71	69	70	71
3	30-35gm	75	2.0	1.10	70	68	63	64
4	35-40gm	90	2.9	2.3	85	82	81	80

PLANT EXTRACT

Sl.No	Animal Wight	Paw Volume	Plant Extract	Inflammatory Agent	Paw Volume			
					15min	30min	45min	1hr
1	20-30gm	78	0.85	0.8	70	69	68	65
2	25-35gm	69	1.0	0.85	69	66	67	65
3	30-35gm	60	1.5	0.90	64	64	63	62
4	35-40gm	80	1.9	1.4	74	72	72	70

STANDARD DRUG

Sl.no	Animal wight	Paw volume	Stand.drug	Inflammatory agent	Paw volume			
					15min	30min	45min	1hr
1	20-30gm	75	1ml	0.2	68	70	62	61
2	25-35gm	70	0.5ml	1.25	67	72	64	54
3	30-35gm	80	0.8ml	1.3	75	73	71	70
4	35-40gm	85	0.85ml	1.35	74	72	70	72

STATISTICAL ANALYSIS

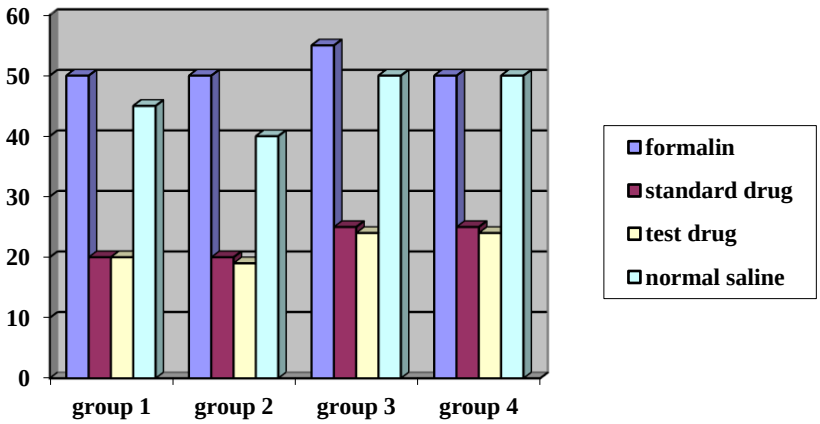

Figure 1: Effect of formalin on nimesulide and plant extract-induced paw edema in mice. The above graph represents the graph values. Between Group 1 (formalin 0.1ml of 1% formalin), Group 2 (formalin + nimesulide 10 mg/kg), Group 3 (formalin + plant extract 200 mg/kg), Group 4 (control-receives only normal saline).

CONCLUSION:

Our study provides valuable insights into the anti-inflammatory potential of Emblica Officinalis fruit extract. Further research is warranted to fully understand its mechanisms of action, assess its safety in humans, and explore its therapeutic utility in managing inflammatory disorders. Our study contributes to the growing body of evidence supporting the therapeutic efficacy of Emblica Officinalis in combating inflammation. By bridging the gap between traditional wisdom and modern science.We have concluded that the test drug competes with the standard drug at dilutions of test drug.

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