A study of Anti-inflammatory activity of fruit of Emblica officinalis (Amla) in Albino rats

Jeevanagi Santoshkumar[1], Meenakshi S Devarmani [2], Manjunath Sajjanar[3,] M. Sakhare Pranavakumar[4], Prashant Dass[4]

[1]Associate professor, Department of Pharmacology M. R. Medical College, Gulbarga, [2]Associate Professor, Department of OBG, M. R. Medical College, Gulbarga, [3]Professor, Department of Pharmacology, S. N. Medical College, Bagalkot, [4]Post Graduates / Tutors, Department of Pharmacology M. R. Medical College, Gulbarga, Karnataka, India.

Abstract

Background: The importance of traditional systems of medicine and of certain traditional medical practices has now been recognized all over the world.

Aim: The present study was designed to study anti-inflammatory activity of fruit of Emblica officinalis in albino rats and compare with standard agent Diclofenac.

Material &Methods: Anti-inflammatory activity 3 methods were used.

Carrageenan induced rat paw edema method (Acute inflammation), in which 5 animals (3groups) received orally 4% gum acacia, Diclofenac sodium and E.officinalis powder respectively, 1 hour before carrageenan injection into right paw. The paw edema volume measured with plethysmograph after 3 hours and percentage inhibition of edema in various groups calculated.

Rexin pellet granuloma method (Chronic inflammation), in which 4 rexin pellets were implanted into dorsum skin of each rat of 3 groups (n=5), which include control, Diclofenac and E.officinalis powder respectively. Rats were daily fed drugs for 7 days and on 8th day rexin pellets were removed after sacrificing animals. Rexin pellets were kept in incubator at 60^{0}C overnight. Pellets were then weighed and percent inhibition of granuloma tissue was calculated.

Leukocyte emigration rat paw edema method ,

Result: Fruit of E. officinalis showed significant anti-inflammatory activity, in both acute as well as chronic model of inflammation comparable to diclofenac.

Conclusion: E.officinalis may be used as potential herbal drug for both acute and chronic inflammation due to their anti-inflammatory property.

Key Words: Emblica officinalis, Anti-inflammatory, Carrageenan, Rat paw edema. Rexin pellet granuloma, Diclofenac.

Introduction

E. officinalis or Phyllanthus emblica (Syn: Amla, Indian Gooseberry) is an evergreen tree which is highly prized in Tropical Asia. The genus is natural to tropical Southeast Asia, particularly in Central and South India. It is commonly cultivated in gardens throughout India and grown commercially as a medicinal fruit [1]. It is among the most important medicinal plants in the Ayurvedic Materia Medica and widely used in Indian medicines for the treatment of various diseases [2]. It is used to treat hemorrhage, diarrhea and dysentery [3]. In folk medicine, all parts of the plant, including fruit, seed, leaf, root, bark and flowers are used in various Ayurveda / Unani herbal preparations [4].The harmacological studies shown that amla fruit was able to lower the lipid level in the liver of rabbit. It was also used as an antimicrobial [5], anti-tumor and analgesic agent [6,7], as well as has shown hypoglycemic activity. E.officinalis leaf extract possesses anti-inflammatory action [8]. Apart from traditional uses, there are several reports in the pharmacological actions of Amla based on modern scientific investigations especially anti-inflammatory action [9], antimicrobial action [10], anti-oxidant action [11], anti-carcinogenic action [12], anti-ulcer genic action [13], anti-diabetic action [14], analgesic action [15], and hepatoprotective action [16]. Inflammation is a complex reaction in vascularized connective tissue, which is elicited by the same exogenous and endogenous stimuli causing cell injury. The term “inflammation” is derived from the Latin literature and the Greek “Phlegmasia”. Although inflammation helps in clearing infection and wound healing, both inflammation and repair have tremendous potential to cause harm. Inflammation may contribute to a variety of disease that are not thought to be primarily due to abnormal host response .For instance, chronic inflammation

Address for correspondence
Dr. Jeevanagi Santohskumar
Associate Professor, Dept of Pharmacology, M. R. Medical College, Gulbarga-585105 Karnataka, India
E- mail: drjeevangi@gmail.com

may play a role in Atherosclerosis, Rheumatoid arthritis, Type -2 diabetes, Alzheimer disease and Cancer[17]. Atherosclerosis, which was earlier thought to be always associated with hypercholesterolemia, has now been proved as an inflammatory disease [18]. Carrageenan induced hind paw inflammation is a neutrophil-mediated acute inflammatory response that produce hind paw swelling, erythema and localized hyperthermia [19]. Free radicals play an important role in the pathogenesis of inflammation [20].The use of substance of plant origin for medical purpose can be said to be as old as mankind itself. The importance of traditional systems of medicine and of certain traditional medical practices has now been recognized all over the world. Today, it is required to have an intelligent and pragmatic approach to evaluate selective drugs of herbal origin. Therefore, it should really matter for Pharmacologists to obtain information from traditional healers, about their remedies and to extract active principles for development into drugs [21]. Keeping in view of the above ideas, the present study has been undertaken to evaluate the effect of E. officinalis powder on the acute and chronic inflammation in albino rats.

Materials and Methods

The present study was conducted in the Department of Pharmacology, Mahadevappa Rampure Medical College, Gulbarga, Karnataka, after taking permission from the Institution Ethics Committee and Animal Ethics Committee of M.R Medical College, Gulbarga.

Drugs used in the study

1. Emblica officinalis: The powder obtained from Phytopharma Ayurveda firm from Kolhapur, Maharashtra. The dose in humans is 6g/day, which is equivalent to 540mg/kg in rats [22].
2.Vehicle: Normal Saline (0.9%), local purchase.
3.Gum Acacia: 4%, 2ml/kg of rat, local purchase.
4.Diclofenac sodium: Crude powder obtained Biocon Pharmaceuticals, Bangalore. Human dose 50mg, which is equivalent to 4.5mg/kg in rats [22].
5.Carrageenan: 1%, 0.1ml sc in the paw of rat is obtained from INCO, Ambala.
6.Plethysmograph: Plethysmograph is obtained from INCO, Ambala.

Animals used in the study

The study was carried out in healthy albino rats of Wister strain (Rattus norvegicus). Body weight of animals was selected between 150-200g each. 30 rats of either sex were used in the study.

The animals were procured from Central Animal House, M.R. Medical College, Gulbarga. Animals were maintained on standard animal diet consisting of Bengal Gram, Wheat, Maize, and Carrot in sufficient quantity for the entire period of the study. Water was given ad libitum.

Study design

Albino rats of either sex weighing 150-200g were used. Total 15 rats were selected and were divided into 3 groups of 5 in each. The rats were obtained from the Central Animal House of M.R.Medical College, Gulbarga. Before starting the study, the animals were allowed to acclimatize to the laboratory environment for 1 week and they were provided with standard diet and water ad libitum as per recommendation of (CPCSEA) "Committee for the purpose of control and supervision of experiments on animals", Government of India (Reg. No.142/99,dated 11-07-1999/CPCSEA.) for laboratory animal facilities [23,24].

Rat Paw edema method

A.Group 1 (Control): 4% Gum Acacia, 2ml/kg.
B.Group 2 (Standard Drug): Diclofenac sodium (4.5mg/kg) in 4% Gum Acacia.
C.Group 3 (Test Drug): E.officinalis powder (540mg/kg) in distilled water.

All the drugs were administered orally followed by constant volume of distilled water after each administration to ensure the entry of drug. One hour after feeding, each rat is anaesthetized with ether and under anesthesia 0.1ml of 1% Carrageenan is injected into sub-plantar region of the hind paw of the rat and the volume of paw is measured by Plethysmograph before and after injection of 1% carrageenan [25]. Volume of edema is recorded at the end of 3 hours after Carrageenan administration. Same procedure was adopted for rats of all the groups (Photograph 1 and Photograph 2).Photograph 1: Carrageenan induced rat paw Photograph 2: Control (Edematous), edema. reated [edema suppressed feet].

percent inhibition of edema in drug treated rats (standard and test drugs) is calculated by using the formula.

Vc= Volume of paw edema in control animals
Vt= Volume of paw edema in drug treated animals.

The dose of the drug under study was calculated by using the dose conversion table.

Table No.1: Dose Conversion table [26].

Rexin pellet granuloma method [22].

Discs of equal size and weight were g each punched out from rexin sheet. Two such discs were stitched together with their rough surface exposed outside and rexin covered surfaces facing each other. Rexin pellets were sterilized using 70% ethyl alcohol. Adult albino rats, 15 in number of either sex weighing about 150 to 200gms, were selected and divided into 3 groups of 5 animals each. The first group served as a control and was given 4% Gum acacia orally. The remaining groups received following drugs in 4% Gum acacia suspension. The dose and route of administration is same as that of rat paw edema method.

All the rats were anaesthetized with ether. The dorsal skin was shaved and applied alcohol to maintain aseptic condition. On either side of midline of dorsal skin, four small incisions of about 1cm length were made. A curved forceps was passed through incisions to make subcutaneous pouch around it. Similarly 4 such pouches were made and a sterilized rexin pellets were implanted into each pouch. An extra rexin pellet was implanted in one animal of each group to be used for histopathological study. The incision was sutured with sterilized cotton thread and tincture benzoin was applied to prevent any contamination.

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All the rats were treated with fixed dose of drugs (as mentioned above) once in every 24 hours for seven days including the day of implantation of pellets. The animals were provided with free excess of food and water. During seven days, the rats were observed for any behavioral changes. On the 8th day, rats were sacrificed with ether anesthesia. The implanted pellets along with granulation tissue were removed. All the pellets were cleaned separately, extraneous tissue removed and dried by incubating in hot air oven at 60^{0} for 24 hrs. Net granuloma formation was calculated by subtracting initial weight of rexin pellet (17mg) from the weights noted [27]. The mean weight of granulation tissue for each group was calculated. The difference in weight of granulation tissue of controlled group and drug treated group was made out. The percent inhibition was calculated by using the following formula.

$$\text{Percent inhibition} = \frac{Wc - Wt}{Wc} \times 100$$

Where,

Wc= Weight of pellets in control group.

Wt=Weight of pellets in drug treated group.

Extra rexin pellet implanted in one rat of each group was removed along with the granulation tissue on the 8th day after sacrificing the rat. The pellets was preserved in 10% formalin, and sent for histopathological examination. The specimens of control, standard, and test groups that included different rexin pellet subcutaneous implants were carefully excised, fixed in 10% buffer formalin, properly grossed and processed for paraffin section. Sections were cut at thickness of 5 microns by a rotary microtome. Slides were stained with standard haematoxylin and eosin stain. Stained sections were evaluated for inflammatory exudate, various inflammatory cells and granulation tissue. Results were correlated with other experimental parameters.

Leucocyte immigration in rat paw edema (28, 29, 30].

Procedure: Four albino rats of either sex weighing 150-200gms were categorized into four batches, as already mentioned in the rat paw edema model with normal (not treated with any drug or Carrageenan). The rats of category 2-4 were given oral feeding as described above. After one hour, rat's category 2-4 was anesthetized with ether, and 0.1ml of Carrageenan was injected into sub-plantar region of right hand paw of rat. 6 hour later, skin of the plantar region of rats from category 1-4 were excised aseptically under ether anesthesia and animals were sacrificed. Excised tissue from each category was preserved in 10% formalin, and sent form histopathological examination.

Histopathological Study

Stained sections were carefully examined for edema, acute inflammatory exudate and various inflammatory cells. Results obtained from the histopathological study were correlated with other experimental parameter.

Statistical analysis

Statistical analysis of experimental data was done by Student's “t” test and ANOVA, (one way analysis of variance).

Results

The results obtained from the standard and test drugs are shown in table 1 and figure 1. The percent inhibition of edema in rats treated with Diclofenac sodium, E.officinalis is calculated with reference to the control group by applying unpaired t test. The percent inhibition of edema at the end of 3 hours with Diclofenac sodium was 63.46%, whereas

with E.officinalis was 38.46%. In the rexin pellet granuloma method, percent inhibition of granuloma formation was determined by weighing the rexin pellets after 7 days of their implantation in the subcutaneous tissue. Diclofenac sodium has shown 63.42% inhibition of granuloma formation and by E.officinalis it was 43.28%. When compared against control, Dicofenac sodium ($p < 0.001$), E.officinalis ($p < 0.01$) showed significant anti-inflammatory activity.

Table 1. Effect of diclofenac sodium and E.officinalis fruit powder on Carrageenan induced rat paw oedema

Groups	Control Group			Standard Group			Test group		
Drugs	4% Gum Acacia			Diclofenac Sodium			E.officinalis		
	Edema Volume (ml)			Edema Volume (ml)			Edema Volume (ml)		
Rat	Baseline	At the end of 3 hours	Difference in volume (ml)	Baseline	At the end of 3 hours	Difference in volume (ml)	Baseline	At the end of 3 hours	Difference in volume (ml)
1	1.05	1.45	0.4	1.09	1.33	0.24	1.14	1.42	0.28
2	1.02	1.55	0.53	1.04	1.22	0.18	1.15	1.56	0.41
3	1.14	1.65	0.51	1.07	1.34	0.27	1.09	1.41	0.32
4	1.12	1.81	0.69	1.02	1.1	0.08	1.05	1.31	0.26
5	1.08	1.57	0.49	1.08	1.29	0.21	1.12	1.49	0.37
Mean			0.52			0.19			0.32
% Inhibition of edema				63.46%			38.46%		
Standard Deviation	0.105			0.073			0.062		
Standard Error	0.047			0.0326			0.028		
*t- value	--			5.78			3.7		
*p- value	--			<0.001			<0.01		
**t- value	5.78			--			3.037		
**p- value	<0.001			--			<0.02		

*- Comparison of Anti-inflammatory effects of standard and test drugs agonist control (unpaired t test).
**-Comparison of Anti-inflammatory effects of test drugs with that of standard (unpaired t test).

Table 2. Effect of diclofenac sodium and E.officinalis fruit powder on Rexin pellet granuloma method.

Group (n=5)	Control Group		Standard Group		Test Group	
Drugs	4% Gum Acacia		Diclofenac Sodium		E.officinalis	
Rats (4 pellets/ rat)	Gain in weight of individual rexin pellet (mg)	Mean weight gain (mg)	Gain in weight of individual rexin pellet (mg)	Mean weight gain (mg)	Gain in weight of individual rexin pellet (mg)	Mean weight gain (mg)
1	40,29,17,18	25.75	11,18,06,10	10.5	14,18,13,15	15
2	21,36,30,22	27.25	9,12,10,15	11.5	15,20,18,13	16.5
3	26,36,25,28	28.75	10,11,10,08	9.75	16,21,21,15	18.25
4	35,39,30,31	33.75	12,13,09,12	11.5	16,19,15,20	17.5
5	34,31,32,37	33.5	09,10,12,14	11.25	21,22,14,12	17.25
Total Mean		29.8		10.9		16.9
% Inhibition of granulation tissue	--		63.42%		43.28%	
Standard deviation	3.58		0.77		1.80	
Standard error	1.6		0.344		0.8	
*t- value	--		11.55		7.51	
*p-value	--		<0.001		<0.001	
**t-value	11.55		--		6.29	
**p-value	<0.001		--		<0.001	

*- Comparison of Anti-inflammatory effects of standard and test drugs agonist control (unpaired t test).
**-Comparison of Anti-inflammatory effects of test drugs with that of standard (unpaired t test).

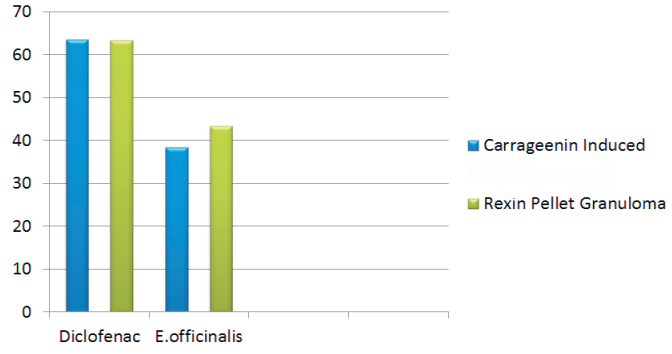


Figure 1: Graph showing results of Carrageenan induced rat paw edema and Rexin pellet granuloma method.

E.officinalis: The Section showed moderate decrease in inflammatory cells like neutrophils, monocytes and eosinophil's in the fields nearest to the pellet. There was a decrease in granulation tissue to about 30-40% in study under various low and high power fields on comparison with the controls. This showed that E.officinalis displayed anti-inflammatory activity but slightly lesser than the standard drug.

Histopathological study

Normal skin: The dermis showed collagenous tissue and subcutaneous tissue showed sub-epithelial glands. There was no edema or inflammatory cells.

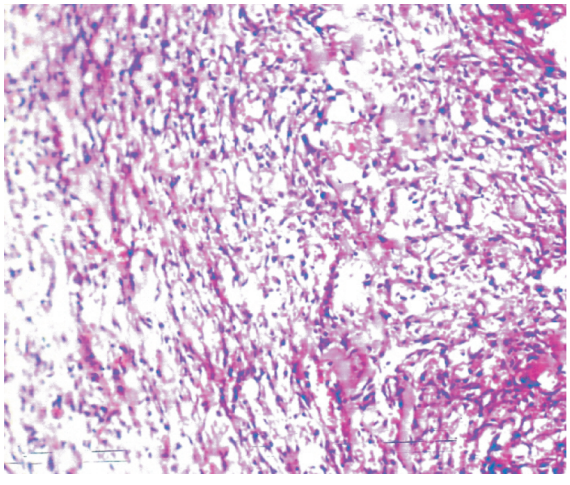
Control: This section shows predominantly neutrophils, few macrophages and dense fibro-collagenous tissue present in the deep. (Photograph 1)

Diclofenac sodium

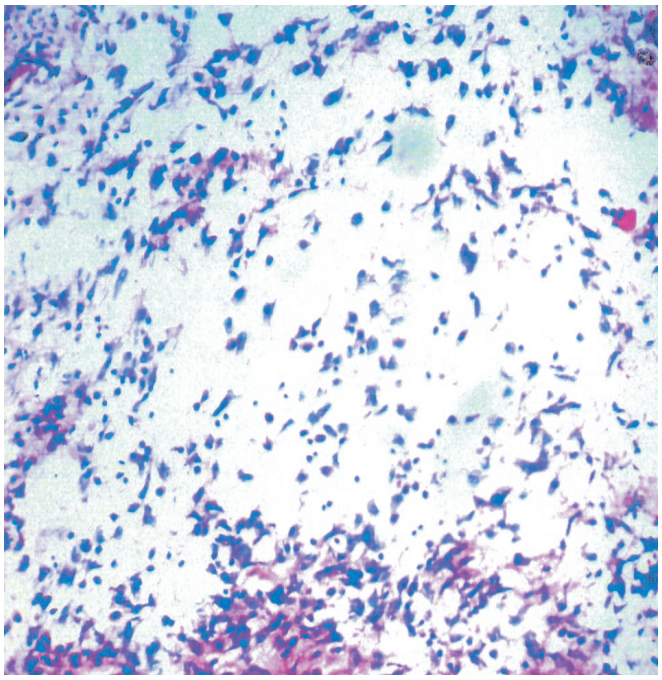
The section showed minimal edema few inflammatory cells like neutrophils and macrophages. There was a decrease in edema and inflammatory cells to around 50-60 %.(Photograph 2)

Emblica officinalis

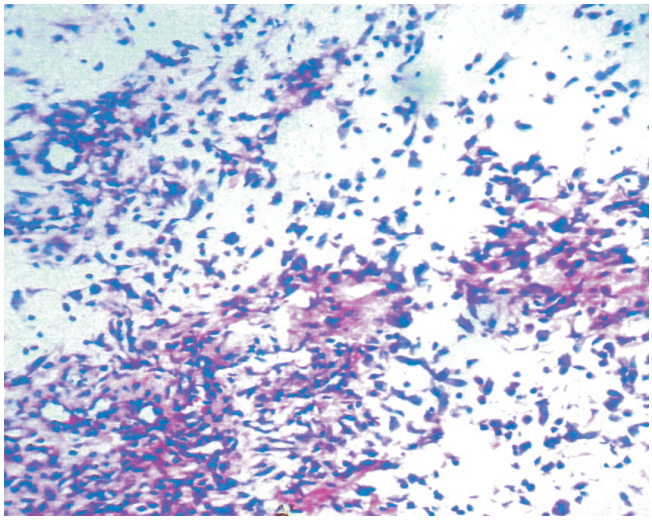
Slides from the groups revealed slightly less edema and inflammatory cells like neutrophils and macrophages as compared to the control group. Edema and inflammatory infiltration was decreased to about 30-40% in both high and low power fields when compared with the control. This indicated that E.officinalis has a slightly lesser anti-inflammatory activity than Diclofenac sodium. (Photograph 3)



Photograph 1. Control showing exuberant granulation tissue, inflammatory cells and fibroblasts.



Photograph 2. Diclofenac shows decrease in granulation tissue, inflammatory cells, and fibroblasts as compared to control.



Photrograph 3. E.officinalis showing moderately decreased granulation tissue, inflammatory cells, and fibroblasts.

Discussion

The present study was planned to investigate the anti-inflammatory effect of E.officinalis fruit powder. The result obtained from this study revealed that E.officinalis fruit powder exhibited significant anti-inflammatory activity in both acute as well as chronic models of inflammation, on comparison with standard drug Diclofenac sodium. According to Asmavi MZ et al [8], they have evaluated the anti-inflammatory activity of E.officinalis aqueous extract on albino rats. Results suggested that aqueous extract of E.officinalis at a dose of 200mg/kg/day, has significant anti-inflammatory activity in Carrageenan induced hind paw edema in rats. Until now no study has been conducted for studying the effects of E.officinalis fruit powder on inflammation. Previous studies have used aqueous and ethanolic extract of E.officinalis and in the present study the effect of E.officinalis fruit powder on chronic inflammation is also studied, which was not done before. Histopathological study of acute and chronic inflammation is also done in the present study, which was not done previously.

The inflammatory process was reported to be associated with the generation of Reactive oxygen species (ROS) [31]. Recently, literature reveals that E.officinalis found to possess the phenolic compounds, i.e. flavonoids, phenolic acids, etc. [32]. Phenolic compounds like Gallic acid, tannic acid are having strong anti-oxidant actions [33]. It is also evident that phenolic compounds obtained from natural source may reduce oxidative stress by free radical scavenging activity [34]. Further, leaf extract of E.officinalis also expressed anti-oxidant action due to the presence of free phenolic constituents [35].

Conclusion

Not many studies have been undertaken to fully evaluate the molecular and biochemical basis of anti-inflammatory action of Amla. Thus, Amla, a commonly used natural product, deserves further evaluation from the stand point of its anti-inflammatory effects in therapy. Today there is wide spread interest in drugs derived from plants. The shortcomings of the drugs available today propel the discovery of new Pharmaco-therapeutic agents in medicinal plants. So emphasis should be laid upon discovery of different active principles in Amla for the control of various inflammatory diseases.

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