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Formulation and evaluation of herbal gel containing *averrhoa carambola* linn fruit extract

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ABSTRACT

Topical drug delivery system used for centuries offering an advantage of delivering drug directly to the site of action for extended period of time at the affected area that mainly acts the related regions. These systems increase contact time and mean resident time of the selected drug. An aim of the present study to focus on the formulation of a gel containing fruit extracts of *Averrhoa carambola* and their evaluation characteristics. The gel was formulated using an aqueous fruit extract of *Averrhoa carambola* using Carbopol 934 and sodium alginate as a gelling agent. The results of preliminary phytochemical investigation showed the presence of phenols, flavonoids, and alkaloids, steroids, terpenoids and carbohydrates. The gel parameters such as pH, Viscosity, spreadability, extrudability were found in satisfied range. The drug content of all prepared gel was found to be (91.25 95.13%w/w). An*in-vitro* drug release results found that, F1 and F4 have shown better percentage release than other formulations, which was taken for anti-microbial and anti-inflammatory activities. An anti-microbial effect of selected formulation has proven active against gram positive and gram negative bacteria than standard. The results showed that an anti-inflammatory effect of formulations containing both gelling agent have shown better reduction in the carrageenan induced paw edema. An optimized formulation promotes better anti-microbial and anti-inflammatory activity than standard. The stability studies proven that there is no significant changes observed in their physical appearance, pH, Spreadability and drug content. And it could be concluded that, prepared formulation containing *Averrhoa carambolafruit*extract gel possess an effective anti-microbial action and anti-inflammatory activity and suitable for topical application and/or treatment for an inflammatory wound area.

INTRODUCTION

Herbal medicine is the oldest form of healthcare known to mankind. Plants have always been an excellent source of drugs and many of the currently available drugs have been derived directly or indirectly from them [1]. Herbal medicines have often retained popularity for chronological and cultural ingredients and are used primarily for treating mild and chronic ailments. India has an ancient heritage of traditional medicines; Materia Medica of India provides lots of information on the custom practices and traditional aspects of therapeutically important natural products [2]. Indian materia includes about 2000 drugs of natural origin almost all of which are derived from different traditional systems and folklore practices. Out of these drugs derived from traditional system, 400 are of mineral and animal origin while the rest are of the vegetable origin [3]. Natural products and especially those derived from higher plants have historically played a pivotal role in the discovery of new pharmaceuticals [4]. World health organization (WHO) estimates that about 80% of the population living in the developing countries relies almost exclusively on traditional medicine for their primary healthcare needs [5]. *Averrhoa*


Fig 1 : *Averrhoa Carambola* Linn Fruit

carambola is a small, attractive, multi stemmed, slow growing evergreen tree with a short trunk or a shrub. It has a bushy shape with many branches producing a broad, rounded crown.Star fruit is believed to have originated in Ceylon and the Moluccas, but it has been cultivated in Southeast Asia and Malaysia for hundreds of years. The perennial herb is commonly grown in Malaysia, Taiwan, Thailand, Israel, Florida, Brazil, Philippines, China, Australia, Indonesia, in the warmer parts of India, Bangladesh and other areas of the world with the same climate. The fruits are green when small and unripe but turn to yellow or orange when matured and ripe. The fruits are fleshy with an oblong shape. The fruits are crunchy, having a crisp texture and when cut in cross-section are star shaped, hence its name. The odor of the fruits resembles oxalic acid and their taste varies from very sour to mildly sweetish or sweetish (6-7). The flesh is light yellow to yellow, translucent and very juicy without fiber. In India, the ripe fruits or its juice are used as anti-pyretic, laxative, appetite stimulant, sialogogue, astringent and antiscorbutic. In Ayurveda, the ripe fruit is considered as digestive, tonic and causes biliousness.

The topical delivery of drugs is an attractive method for local and systemic treatments and is commonly used in the treatment of inflammatory conditions like dermatological diseases and musculoskeletal injuries [9-10]. Topical application has many advantages over the conventional dosage forms, especially to avoid some serious systemic adverse effects [11].When the drug is delivered topically it can penetrate deeper into skin and hence give better absorption. Topical preparation prevents the metabolism of drug in the liver, avoids gastrointestinal disorders and the risks and inconveniences of intravenous therapy, and avoids the risks associated with the varied conditions of absorption, like pH changes, presence of enzymes, and gastric emptying time. Furthermore, the bioavailability of the drug is increased and its action occurs directly at the action site [12-13]. A wide variety of pharmaceutical dosage forms can be used in the delivery system for topical drugs [14-15]. The topical delivery with gels can increase the resistance time of the drug on the skin and improve the delivery and release of the substance by increasing the residence time at the site[16].

MATERIALS AND METHODS

Materials

Plant Collection and Authentication

Fresh fruit of *Averrhoa carambola* (oxalidaceae) were collected from local areas of Kottakal, Kerala and was authenticated by Dr. A. K Pradeep, Department of Botany, University of Calicut, Calicut, and a voucher specimen was deposited (No.148258).

Chemicals

Triethanolamine, propylene glycol and disodium edetate were purchased from Sigma-aldrich, Bengaluru. Carbopol 934 and sodium alginate were obtained Gift sample from Dr.Reddy laboratory Pvt. Ltd. Hyderabad, solvents in analytical grade.

Preparation of PlantFruit Extract

The foreign, earthy matter and residual materials were removed carefully from the fruits and then cleaned then its cuts in to small pieces and allowed to dry. The dried fruits were grinded in to coarse powder and allowed to extract with methanol by cold maceration process for 7 days. Then it was filtered and concentrated under reduced pressure in IKA Rotary evaporator (Model No RN 10 digital V, ILMAC Germany) at 40 °C and stored at 4-8°C for further use.

Percentage Yield

In order to analyses the percentage plant yield extract, the extracts of evaporated dried extracts based on dry weight basis were calculated by using gravimetric analysis as the following equation: Percentage yield (%) = (W1 × 100) / W2, Where W1 is the weight of the extract after the solvent evaporation and W2 is the weight of the initial powder used to extract [17].

Phytochemical Investigation

An alcoholic extract was subjected to qualitative chemical investigation. The suitable procedures were chosen to detect the presence of various phytochemical constituents in the extract. Most important of these bioactive constituents of plants are steroids, terpenoids, carotenoids, flavanoids, alkaloids, tannins, phenols, carbohydrate and glycosides. Phytochemicals are used as templates for lead optimization programs, which are intended to make safe and effective drugs [18].

Preparation of Gel formulation

Accurately weighed Carbopol 934 was taken and dispersed in 50 ml of distilled water and kept a side to swell and then stirred using mechanical stirrer for 30 min. Take 5 ml of propylene glycol and required quantity of extract. 5ml propylene glycols is taken in another beaker and add weighed quantity of propyl and methyl paraben to it and stirred properly. After all Carbopol dispersed, adequate quantities of prepared extract were added with constant stirring. Finally volume made up to 100 ml by adding distilled water and triethanolamine added drop wise to the formulations for adjustment of skin pH (6.8-7) and obtained gel at required

Table 1 : Composition of Topical herbal gel formulations with Carbopol 934 and Sodium alginate

Ingredients	F1	F2	F3	F4	F5	F6
Fruit extract(Averrhoa carambola) (gm)	2	2	2	2	2	2
Carbopol 934 (gm)	0.5	0.3	0.1	-	-	-
Sodium alginate (gm)	-	-	-	0.5	0.3	0.1
Disodium EDTA (gm)	0.005	0.005	0.005	0.005	0.005	0.005
Methyl paraben (gm)	0.25	0.25	0.25	0.25	0.25	0.25
Propyl paraben (gm)	0.25	0.25	0.25	0.25	0.25	0.25
Proplene glycol (ml)	2	2	21	2	2	2
Deminarilized water (ml)	Qs	Qs	Qs	Qs	Qs	Qs
Triethanolamine (ml)	1	1	1	1	1	1

consistency. Six topical Gel formulations (Table 1) were prepared using *Averrhoa carambola linn* fruit extract. F1 to F3 formulations using gel base of Carbopol 934 and F4 to F6 formulations were prepared using gel base of sodium alginate (19).

Estimation of active constituents

Each formulation (1g) was taken in a 50 ml volumetric flask and made up to volume with methanol and shaken well to dissolve the active constituents in methanol. The solution was filtered through whatman filter paper and 0.1 ml of the filtrate was pipette out and diluted to 10ml with methanol (20). The content of active constituents in the extracts was estimated spectrophotometrically by using standard curve plotted at 275 nm.

Extrudability

A closed collapsible tube containing about 20 g of gel was pressed firmly at the crimped end and a clamp was applied to prevent any roll back. The cap was removed and the gel was extruded. The amount of the extruded gel was collected and weighed. The percentage was calculated [21].

pH measurement

pH measurement of gel was carried out using a digital pH meter by dipping the glass electrode completely into the gel system to cover the electrode. The measurement was carried out in triplicate and the average of the three readings was recorded [22].

Viscosity

Viscosity determined using Brookfield viscometer (S-62, model LVDV-E) at 25°C with a spindle speed of the viscometer rotated at 12 rpm. The assembly was connected to a thermostatically controlled circulating water bath maintained at 25°C. The formulation whose viscosity was determined, added to a beaker covered with thermostatic jacket. Spindle was allowed to move freely into the gel and the reading was noted [22].

Spreadability

Two sets of glass slides of standard dimensions were taken. The herbal gel formulation was placed over one of the slides. The other slide was placed on the top of the gel, such that the gel was sandwiched between the two slides in an area occupied by a distance of 7.5 cm along the slides. Adequate weight equivalent of gelwas placed on the upper slides, so that the gel between the two slides was pressed uniformly to form a thin layer. The weight was removed and the excess of gel adhering to the slides was scrapped off. The two slides in position were fixed to a stand without slightest disturbance and such a way that only upper slides to slip off freely by the force of weight tied on it. 20 g was taken and tied to the upper slide carefully. The time taken for the upper slide to travel the distance of 7.5 cm and separated away from lower slide under the influence of weight was noted. The experiments were repeated and mean time was taken for calculation [23]. Spreadability calculated using following formula: $S = m \times l/t$, where, S= spreadability, m-weight tied to upper slides (20 g), l-length of the glass slide (7.5 cm), t- time taken in sec.

In vitro permeability study

In vitro diffusion studies were carried out using Franz diffusion cell. The diffusion cell apparatus fabricated locally as open-ended cylindrical tube with 3.7994 cm^2area and 100 mm height having a diffusion area of 3.8 cm2. Phosphate buffer (pH 7.4) was used as receptor media. Rat abdominal skin was used as dialysis membrane. The skin was tied to the diffusion cell (donor cell) such that the s*tratum corneum* side of the skin was in intimate contact with the release surface of the formulation in the donor cell. Isotonic phosphate buffer solution, pH 7.4 (100 ml) was added to a donor compartment prior to be mounted on the diffusion cell. A weighed quantity of formulation equivalent to 1g of gel was taken on to the rat skin and was immersed slightly in 100 ml of receptor medium, which was continuously stirred. The entire system was maintained at 37±1 ºC. An aliquot of 5 ml was withdrawn at specific time intervals up to 6 hrs, and was estimated spectrophotometrically at 275 nm. After each withdrawal, diffusion medium was replaced with an equal volume of fresh diffusion medium. The cumulative percent release was calculated for each time interval[24].

Anti-Microbial study

Anti-bacterial activities of plant extract gel were assessed using standard well-diffusion method. An anti-bacterial assay, individual drug extract using nutrient broth (by broth dilution), and gel formulation using nutrient agar (by agar well diffusion method). The formulated Gels were compared with marketed Gentamycin (standard) and an estimated antimicrobial activity[25].

Microbial growth

Nutrient agar media was used in microbial growth study. In this method the blank and sample petriplates were used and gel sample were aseptically transferred on the sample plates in across pattern. The microbial growth was observed.

Antimicrobial Activity

Microorganisms

An anti-bacterial organism such as*Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*, *Escherisia coli* were used in these studies. All bacterial strains were grown and maintained on nutrient agar slants for 24 hrs and Candida albicans were also grown on Sabouraud's Dextrose Agar slants for 48hrs to 72 hrs. All microorganisms were confirmed by staining technique.

Determination of Zone of inhibition

It was carried out using selected formulations by cup-plate method and prepared plates were incubated for 24 hrs at 37ºC and evaluated an antibacterial activity using nutrient agar against gram negative and gram positive microorganisms.

Skin irritation studies

Wistar rats of either sex weighing 150-200gm were used this test. The hairs were removed from ratson 3 days prior experiment. The gels containing extracts were used on test animal. Only Gel base was applied on animal skin taken as control. An animal were treated with gel,on daily up to seven days and treated intact skin was examined visually for erythema and edema[26].

Screening of an Anti-inflammatory Activity - Animal Used

Healthy Wistar albino rats (either sex) - weighing 150-200 gm and 60 days old were maintained in an identical laboratory conditions (25º- 30ºC temperature and relative humidity of 55-65 % with alternate light and darkness 12 hrs each) and fed with commercial pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. All experimental procedures described were reviewed and approved by institutional animal ethical committee (IAEC approval no: DAMCOP/IAEC/058).

Carrageenan induced paw edema in Wistar albino rats

The Wistar albino rats (150-200 g) either female or male are divided in to 4 groups.Each group comprising of six animals.

Carrageenan induced rat paw edema studies

An edema was induced by injecting 0.1 ml carrageenan (1% w/v) in normal saline in to the sub plantar region of the left hind paw. It was evaluated according to the method described by Winter et al., (1962). Swelling of carrageenan injected foot was measured at 0, 1, 2and 3 hrs using Plethysmometer (UGO Basile, Italy). Animals were treated with test extract before carrageenan injection. Measurement was carried out immediately before and 3hrs following carrageenan injection. Percent inhibition of test drugs was calculated in comparison with vehicle control (100%) [27].

Stability Study

The stability study was performed as per ICH guidelines. The formulated gel were filled in thecollapsible tubes and stored at different temperatures and humidity conditions, viz.25° C ± 2°C/ 60% ± 5% RH, 30° C ± 2°C/ 65% ± 5% RH, 40° C ± 2°C/ 75% ± 5% RH for a period of threemonths and studied for appearance, pH, viscosity and Spreadability[28].

RESULTS

Organoleptic Properties

Table 2 : Organoleptic properties of the plant extracts

Description	Crude drug	Ethanolic extract
Colour	yellowish	Dark green
Odour	Characteristic	Characteristic
Taste	Bitter	Bitter

Extraction of Plant material

The plant fruits were extracted by maceration technique and the percentage yield of aqueous fruit extract was found to be 2.5%.

Table 3 : Preliminary phytochemical analysis

Plant Constituents	Tests	Observation
Alkaloids	Mayers test	+
	Dragendroffs	+
	Hagers test	+
Flavanoids	Lead acetate	+
	Zinc Hcl	+
	Shinoda	+
Tannins and phenols	Gelatin	+
	Vanillin	+
Carbohydrates	Molishes	+
	Fehlings	+
	Benedict	+
Glycosides	Balget	-
	legal	-
Steroids	Steroid test	+
Terpenoids	Terpenoids test	-

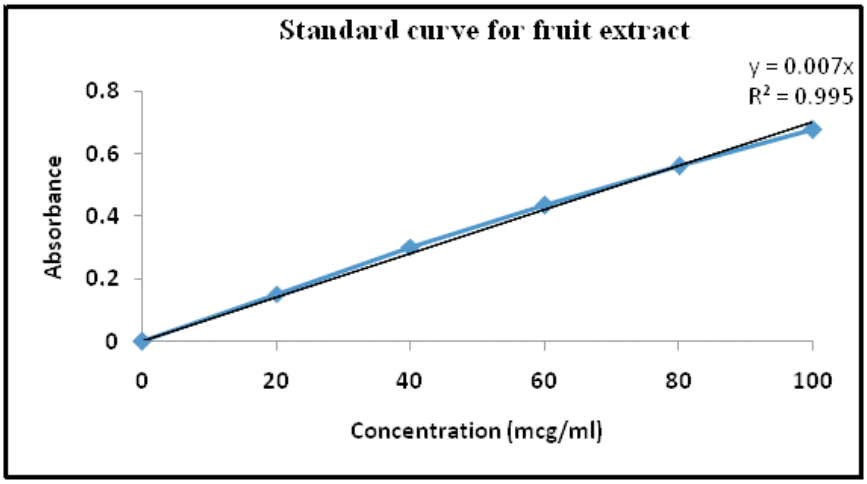

Fig 2 : Standard curve of *Averrhoa carambola* fruit extract

Table 4 **:** Evaluation parameters of gel formulation made with Carbopol 934 & Sodium salicylate

Forms. code	Nature of gel	pH	Viscosity (cpm)	Spreadability (g.cm/sec) M = (l/t)	Extrudability	Percentage drug content (%)
F1	Whitish smooth &translucent	6.9	4120	25±0.49	96.76±0.007	95.2±0.007
F2		7.0	4250	30±0.53	84.12±0.007	93.4±0.110
F3		7.0	3980	30±0.53	90.54±0.006	92.5±0.211
F4	Greenish smooth & translucent	6.0	4120	30±0.53	85.20±0.004	93.3±0.115
F5		6.9	4210	37± 0.66	84.12±0.007	92.5±0.271
F6		6.8	3990	25±0.49	83.68±0.007	91.2±0.038

Results are mean SD of three trials (n=3)

Preparation of Standard Solution of Extract

Standard solution of extract was prepared in different concentration which shown in Figure 2. The absorbance was measured at 275 nm against ethanol as blank and then plotted the standard curve of fruit extract.

Extrudability

(>90% extrudability: excellent, >80% extrudability: good, >70% extrudability: fair)

The extrudability reflects the capacity of gel, to get rejected in uniform and desired quantity when the tube is squeezed. From results, all formulations shown good excellent extrudability, which depends on the polymer concentration.

In vitro diffusion studies

An*in vitro* release profile of fruit extract gel from various formulations was depicted in Figure 3.

At 1hr, F1 formulation showed maximum drug release with 31.46% and lowest concentration of shown by F6. At 2 hrs of diffusion study, formulation F1 again showed maximum release with 38.89% and lowest release was observed in F6 with 22.57%. After 6 hrs, formulation F1 showed consistent release of drug with 83.65%. It might be due to the higher concentration of gelling agent.

Optimization:

The batches were optimized by checking studying physical evaluation to their pH, viscosity, Spreadability and Extrudability. The evaluation parameters of all batcheswere optimized. Overall F1(Carbopol based gel)and F4 (Sodium alginate based) gelformulation was selected for anti-microbial and anti-inflammatory study.

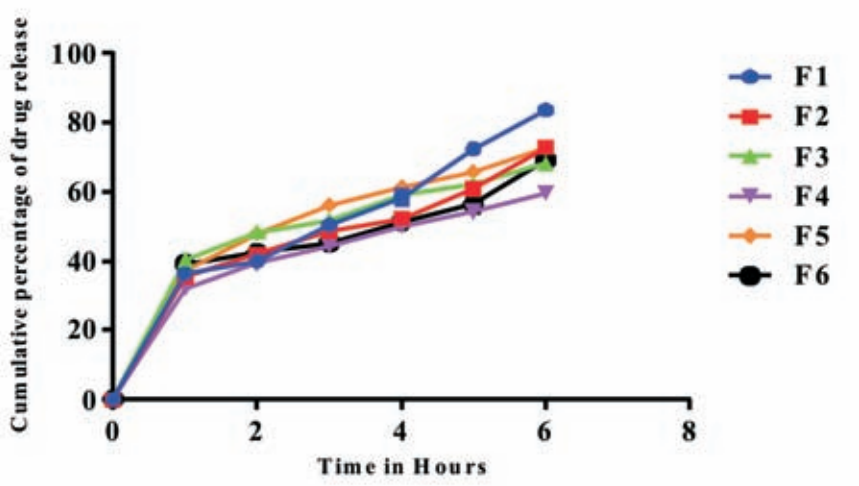

Fig 3 : *In vitro* drug release from various gel formulations

Table 5 : Measurement of Zone of Inhibition

Microorganisms	Zone of Inhibition (mm)		
	Formulations (F1)	Formulations (F4)	Standard
Bacillus subtitles	23	23	47
Escherichia Coli	20	20	41
Staphylococcus aureus	25	25	47
Pseudomonas aeroginosa	25	25	52

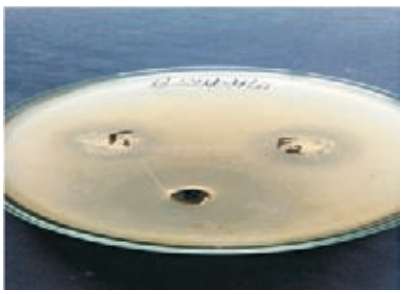

Fig 4 **:** Measurement of Zone of Inhibition using *Bacillus Subtitles*

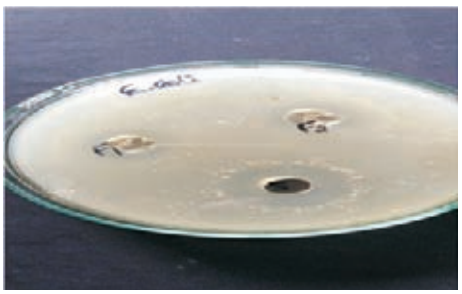

Fig 5 : Measurement of Zone of Inhibition using *Escherichia Coli*

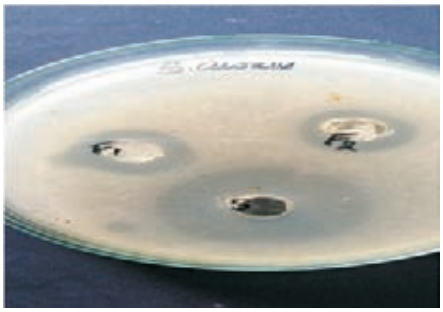

Fig 6 : Measurement of Zone of Inhibition using *Staphylococcus aureus*

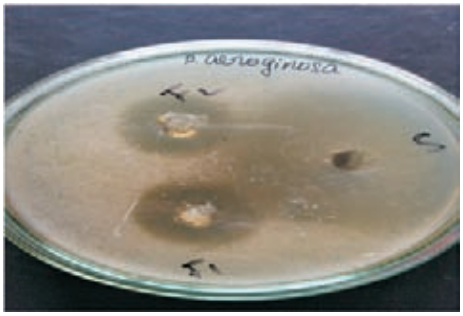

Fig 7 : Measurement of Zone of Inhibition using *Psuedomonas aeroginosa*

Antimicrobial study

Well Diffusion Method

Selected formulations were active against gram positive and negative micro-organism. From the results it observed that, the formulations have much more active against *psuedomonas aeroginos*a and *staphylococcus aureus* bacterias based on their zone of inhibitions.Formulation containing both extracts showed significant zone of inhibition and exhibit maximum inhibition ranging from 20 to 25 mm than marketed Gentamycin preparation used as a standard.

Skin irritation test

The prepared gel was evaluated for skin irritant effect, where no erythema or edema was observed for all the formulations, even after 10 days of study, indicating that the prepared Gelformulation was found to be safe.

Anti-inflammatory activity

Table 7 : Effect of Formulations on Carrageenan induced paw edema in rats

Groups	0 min	30 min	60 min	120 min	180 min	240 min
Negative control	4.74±0.008	4.96±0.008	4.75±0.008	4.59±0.008	4.52±0.011	4.43±0.025
Group 1 (F1)	4.25±0.008 ***	4.35±0.011 ***	3.98±0.008 ***	3.65±0.008 ***	2.95±0.027 ***	2.22±0.008 ***
Group 2 (F2)	4.20±0.011 ***	4.29±0.011 ***	3.95±0.008 ***	3.52±0.008 ***	2.85±0.014 ***	2.20±0.008 ***
Standard	4.49±0.008 ***	4.85±0.008 ***	3.86±0.008 ***	3.34±0.008 ***	3.01±±0.011 ***	2.35±±0.020 ***

Data expressed as Mean ±SEM as compared with control ***P <0.001value calculated by comparing with control by ANOVA followed by Tukey-kramer multiple comparison test.

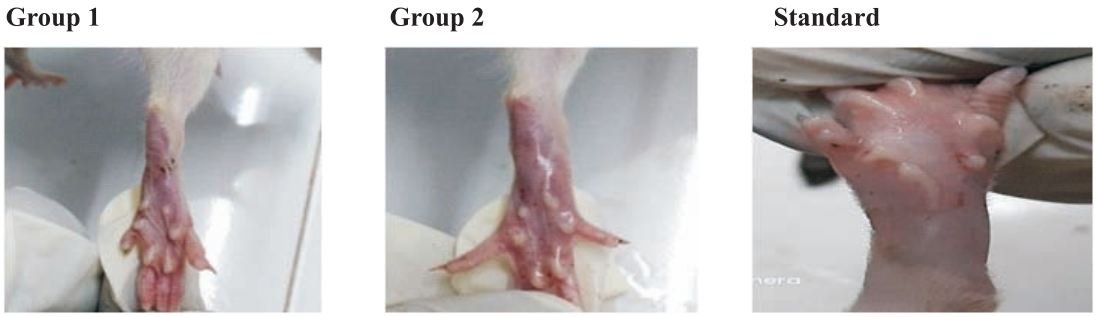

Fig 8 : Effect of Formulations on Carrageenan induced paw edema in rats(at 240 min)

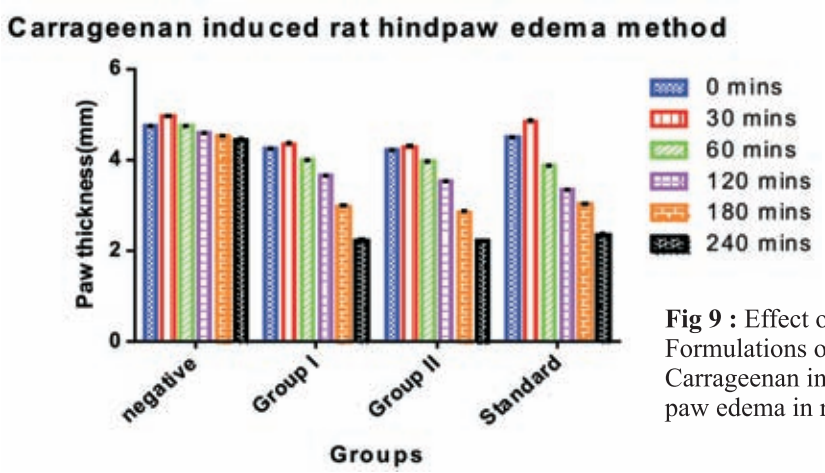

Fig 9 : Effect of Formulations on Carrageenan induced paw edema in rats

Table 8 : Effect of Formulations in percent paw thickness of inhibition

Groups	Percentage of inhibition (%)					
	0 min	30 min	60 min	120 min	180 min	240 min
Negative control	–	–	–	–	–	–
Group 1	7.56	6.45	32.24	35.28	39.37	49.85
Group 2	8.71	8.29	32.45	38.21	42.58	48.75
Standard	3.28	5.23	28.34	30.57	34.10	47.65

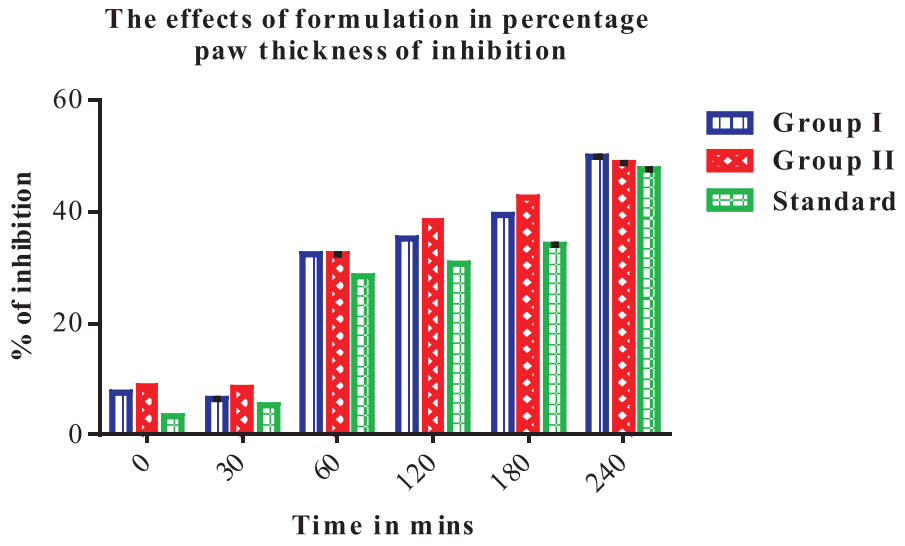

Fig 10 : Effects of formulations in percent paw thickness of inhibition

F1 and F4 was evaluated an anti-inflammatory activity using carrageenan-induced rat paw edema. The result showed that an anti-inflammatory effect have shown better reduction in the carrageenan induced paw edema than standard. So, it can be concluded F1 showed significant results than F4 and control.

Stability Study

It was observed that, there was nocolor fading during tested periods. The pH of F1 formulation was not affected and found within the range of 6.9 -7.1. Spreadability was found in effective range. Drug content during the stability period not changed much found between 95.21-93.24% at 40°C.Hence, it's concluded that, there was no significant changes observed and indicates that, prepared gel was stable during such periods.

DISCUSSION

Herbal gel was prepared and subjected to evaluation of various parameters. The gel was greenish in colour with a translucent appearance and cooling sensation throughout the evaluation Period. All formulations were found to be neutral (pH 6.8 to 7.0) and drug content was found to be in the range of (91.25 -95.13% w/w). On the physical evaluation of all formulations were found to be optimum in terms of Gel consistency, viscosity, Spreadability, and extrudability. Extrudability was excellent, while Spreadability was less variant after performing stability studies.

Based on *in-vitro p*ermeability release F1 & F4 exhibited released within 6 hours of permeability study and F1 formulation showed better release. Anti-microbial study was carried out in F1 and F4 formulation by agar well diffusion method. The results found that both formulations shown better activity against gram negative and gram positive bacteria's. When compared to other bacteria's both formulations were active against pseudomonas aeroginosa and *staphylococcus aureus* bacteria's. All formulations were nonirritant and did not show any skin toxicity. An anti-inflammatory effect produced after topical administration of herbal gel formulation on Carrageenan-induced hind paw oedema exhibited a high degree of reproducibility in both formulation (F1 and F4). Stability studies revealed that there was no significant difference in the physical and chemical parameters and were found be stable in the said period.

Table 9 : Stability studies of Gel formulation (F1)

Duration in (days)	At 25±2°C/60±5%RH				At 40±2°C/75±5%RH			
	Physical appearance	pH	Spread ability (g.cm/sec)	Drug content (%)	Physical appearance	pH	Spread ability (g.cm/sec)	Drug content (%)
0	Greenish smooth &translucent	6.9	25±0.49	95.20± 0.112	Whitish smooth &translucent	6.9	25±0.49	95.21± 0.02
30	Whitish smooth &translucent	6.9	25±0.59	94.35± 1.01	Whitish smooth &translucent	6.9	25±0.19	94.38± 0.19
60	Whitish smooth &translucent	6.9	25±0.09	93.20± 0.21	Whitish smooth &translucent	7.1	24±0.63	93.25± 0.62
90	Whitish smooth &translucent	7.1	24±0.112	93.10± 0.52	Whitish smooth &translucent	7.2	24±0.041	93.24± 0.118

CONCLUSION

In the recent years, topical drug delivery has been used extensively to impart better patient compliance. Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Hence; Herbal formulations have growing demand in the world market. In this research work, good attempt was made to establish herbal gel containing *Averrhoacarambola linn* fruit extract at various concentrations using Carbopol 934 and sodium alginateas as gelling agent. The data presented in this study demonstrated that *Averrhoacarambola linn* fruit extract in form of gel possess significant topical anti-inflammatory properties, supporting their traditional use of treatment. Overall, it can be concluded that, the herbal medicine has become an item of global importance both medicinal and economical. Although usage of these medicines has increased, their quality, safety and efficiency are serious concerns in industrialized and developing countries.

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