EXPLOITATION OF *MANGIFERA INDICA* GUM AS NOVEL NATURAL GELLING AGENT IN THE DESIGNING OF GEL FORMULATIONS

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Running title: Evaluation of *Mangifera Indica gum* as gelling agent

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Summary

Natural gums and mucilages have been extensively explored as pharmaceutical excipients. These gums are biocompatible, cheap and easily available. The gum from the *Mangifera indica* tree (Family Anacardiaceae) was extracted by using water as solvent and precipitated using acetone as non-solvent that yield a high proportions of (35%) of gum. Gum extracted from *Mangifera indica* were subjected to toxicity studies for its safety and preformulation studies for its suitability as a gelling agent. To study the gelling properties, gels were prepared using aceclofenac as model drug. Eight batches of drug loaded gels with concentration of gum corresponding to 2.0,2.5,3.0, 3.5,4.0,4.5,5.0 and 5.5%w/w were formulated by using glycerin as wetting agent and methyl paraben as preservative. The gels were evaluated for drug content, viscosity determination, *in vitro* permeation (across dialysis membrane), skin irritation and stability tests. The gels prepared with 4.5% of gum were found to be ideal and comparable with a commercial preparation. The prepared gels did not produce any dermatological reactions and were well tolerated by the guinea pig. Stability study revealed that the gel formulations were physically stable and has no syneresis. Briefly, it could be concluded that the *Mangifera indica* gum can be used as a pharmaceutical excipient in gel formulations; it has the potential to also replace some synthetic gelling polymers upon further modifications.

Key words: *Mangifera indica, in vitro* permeation, gel, aceclofenac, viscosity.

Introduction

The traditional use of excipients in drug formulations was to act as inert vehicles to provided necessary weight, consistency and volume for the correct administration of the active ingredient, but in modern pharmaceutical dosage forms they often fulfill multi-functional roles such as modifying release, improvement of the stability and bioavailability of the active ingredient, enhancement of patient acceptability and ensure ease of manufacture. New and improved excipients continue to be developed to meet the needs of advanced drug delivery systems.

In recent years, plant derived polymers have mucilages can occur in high concentrations in different evoked tremendous interest due to their diverse pharmaceutical applications such as diluent, binder, disintegrant in tablets, thickeners in oral liquids, protective colloids in suspensions, gelling agents in gels and bases in suppository[1]; they are also used in cosmetics, textiles, paints and paper-making[2]. These polymers such as natural gums and mucilage are biocompatible, cheap and easily available and are preferred to semi synthetic and synthetic excipients because of their lack of toxicity, low cost, availability, soothing action and non irritant nature[3-6]. Demand for these substances is increasing and new sources are being developed. India, because of its geographical and environmental position, has traditionally been a good source for such products among the Asian countries. Still, large quantities are imported from Europe to meet increasing demand.

Gums are high molecular weight polysaccharides which are formed from sugar and uronic units. Gums are hydrophilic in nature and may be classified as natural, semi-synthetic or modified and synthetic. Natural gums including acacia, ghatti, karaya, locust bean, albizia, khaya, guar, tragacanth and xanthan, are obtained as exudates or extractives from the bark of stems, branches and roots of various plants. Plant families notable for the production of gums are Anacardiaceae, Combritaceae, Meliaceae, Rosaceae and Rutaceae[7]. Various reasons have been advanced for the production of gums by plants, including: as products of normal plant metabolism; as a protective mechanism against a pathological condition afflicting the plant; and as a consequence of infection of the plant by microorganisms.

The Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) possess anti-inflammatory, analgesic and antipyretic activities. The Indian drug industry is always ready to cater to the needs of medical professionals by developing combinations of various kinds of drugs that are capturing substantial market share. Aceclofenac is a Diclofenac derivative of the Non–Steroidal Anti-InflammatoryDrug[8-11,] which is chemically,(2-[2-[2-(2,6 dichlorophenyl)aminophenyl] acetyl]oxyacetic acid)[12-13]. Aceclofenac exhibited potent Anti- Inflammatory Analgesic activity and is widely prescribe for the treatment of osteoarthritis, rheumatoid arthritis, acute lumbago, and dental pain condition[14]. Aceclofenac is well tolerated, with most adverse events being minor and reversible and affecting mainly the G.I system. Although the incident of gastrointestinal adverse events with Aceclofenac was similar to that comparator NSAID in individual clinical trial withdrawal rate due to these events were significantly lower Aceclofenac than with Ketoprofen and Temoxicam. Other adverse effects which are not common such as dizziness (1%), vertigo (0.3%) and tremor

For centuries, the Mango tree (Scientific name: *Mangifera indica*, Family: Anacardiaceae) has been an integral part of life in India. Each and every part of the tree (bark, leaves, root and kernel seed fruit) serves a certain purpose, for instance, as diuretic, astringent, aphthous stomatitis, diabetes, asthma, diarrhea, urethritis, dysentery, scabies and other parasitic skin diseases[15].

During earlier study in our laboratory, the disintegrating properties of *Mangifera indica* gum were evaluated[16]. Literature survey reveals that comprehensive physicochemical characterization and pharmaceutical application of the *Mangifera indica* gum (MIG) as gelling agent in pharmaceutical formulation has not been reported yet. Hence the present work was attempted to evaluate gelling properties of gum extracted from trees (injured site) of *Mangifera indica*.

Materials and Methods

Materials

Aceclofenac was obtained as gift sample from Aristo Pharmaceuticals Ltd, Mumbai, India. Mango gum resin was collected from the incised trunk of *Mangifera indica* in Ankola region (Uttar Kannada District). All other materials, excipients, solvents and reagents were either analytical or Pharmacopoeial grade and they were procured from S.D.Fine Chemicals Mumbai.

Methods

Extraction of *Mangifera Indica* Gum[17-18]

The mango gum resin gum was collected from *Mangifera indica* trees (injured trunk site). It was dried, ground, and passed through sieve no 80. Dried gum (15 g) was stirred in distilled water (300 ml) for 6-8 h at room temperature. The supernatant was obtained by centrifugation. The residue was washed with water and the washings were added to separate supernatant. The procedure was repeated four more times. Finally the supernatant was made up to 500 ml and treated with twice the volume of acetone by continuous stirring. The precipitated material was washed with distilled water and dried at 50-60°C under vacuum. The dried gum was pulverized using a pulverizer and stored in tightly closed container.

Evaluation of Toxicity

Toxicity studies were carried out according to the method of Knudsen and Curtis[19]. The animals used in the toxicity studies were sanctioned by the Institute Animal Ethical Committee (Approval No: KLECP/IAEC/45/2010-11). The male albino rats of Wistar strain weighing 160-200 g were divided into different groups comprising of six animals each. The control group received normal 0.5%CMC solution (20ml/kg i.p). The other groups received 500, 1000, 2000, 3000, 4000 and 5000 mg/kg of MIG suspension in normal saline orally. The animals were observed continuously for the behavioral changes for the first 4 hours and then observed for mortality if any for 72h. Since no mortality, no toxic manifestations were observed and behavioural pattern was unaffected. In chronic toxicity studies, 22 animals were used, divided in to two groups, 6 as control and 16 as test animals. In the test group a dose of 500 mg/kg was administered daily for a period of 30 d. body weights were recorded for both the groups at an interval of 10d. And at the end of 30 days, hematological and biochemical parameters were studied in both the groups and after 30 days of chronic toxicity study the animals were scarified and subjected to histopathological studies.

Physicochemical characterization of mucilage[20-22]

The physicochemical properties such as solubility, swelling index, ash values, loss on drying, precompression parameters and microbial load of the MIG were determined according to official Procedures. The following evaluation parameters were carried out as per the procedures described below.

Solubility

The separated gum was evaluated for solubility in water, acetone, chloroform, methanol, ether and ethanol in accordance with the British Pharmacopoeia specifications.

Determination of swelling index

Swelling characteristics of the separated MIG powder was studied in different media such as 0.1 N hydrochloric acid, pH 7.4 phosphate buffer and distilled water. The swelling index is the volume in ml occupied by 1 g of drug; including any adhering gum after it has been swollen in an aqueous liquid for 4 h. The swelling index of MIG powder was determined according to the British Pharmacopoeia method. 1g of MIG powder was taken in a 25 ml ground glass stoppered cylinder graduated over a height of 120 to 130 mm in 0.5 divisions. To this 25 ml of respective medium was added and this was shaken vigorously every 10 m for 1 h and then allowed to stand for 24 h. The volume occupied by the MIG powder was measured.

The swelling index was computed using the equation

S = V2/V1.
Where; S = Swelling index
V1 = Volume occupied by the gum prior to hydration
V2 = Volume occupied by the gum after to hydration
The test was carried out in triplicate and the average value of swelling index was recorded

Loss on drying
As the inherent moisture in MIG powder/excipients may influence the stability of the tablet dosage form containing moisture sensitive drugs, moisture content of the separated mucilage was detected by loss on drying method. The sample (1 g) was heated at 105^{o}C until constant weight in a hot air oven and percentage loss of moisture on drying was calculated using the formula,

LOD (%) = (weight of water in sample/weight of dry sample)×100.

Total ash
The total ash was determined by placing 3 g of the ground air-dried material in a crucible, spreading the material in an even layer and igniting it by gradually increasing the temperature to 550^{o}C until it is white, indicating the absence of carbon. The crucible was cooled in a desiccator, weighed and the content of total ash in mg per g of air-dried material was calculated.

Acid Insoluble ash
Acid-insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid and igniting the remaining insoluble matter. To the crucible containing the total ash, 25 ml of hydrochloride acid was added, covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 ml of hot water this liquid was added to the crucible. The insoluble matter on an ash less filter paper was collected and washed with hot water until the filtrate is neutral. The filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. The residue was allowed to cool in a desiccator for 30 min, weighed without delay and the content of acid insoluble ash in mg per g of air-dried material was calculated.

Microbial load
Microbial count for separated MIG powder was performed as outlined in Indian Pharmacopoeia-1996 for total aerobic microbial count using plate count method. The plate count for bacteria and fungi were measured.

pH determination
This was done by shaking a 1%w/v dispersion of the sample in water for 5 min and the pH determined using a pH meter (Elico, Hyderabad). The data presented here is for triplicate determinations.

Angle of repose
The static angle of repose, a, was measured according to the fixed funnel and free standing cone method. A funnel was clamped with its tip 2 cm above a graph paper placed on a flat horizontal surface. The powders were carefully poured through the funnel until the apex of the cone thus formed just reached the tip of the funnel. The mean diameters of the base of the powder cones were determined and the tangent of the angle of repose calculated using the equation:

Tan a = 2h/D
The data presented here is for triplicate determinations.

Bulk and Tapped densities
2 g quantity each of the powder sample was placed in a 10ml measuring cylinder and the volume, V_0, occupied by each of the samples without tapping was noted. After 100 taps on the table, the occupied volume V_{100} was read. The bulk and tap densities were calculated as the ratio of weight to volume (V_0 and V_{100} respectively). The data presented here is for triplicate determinations.

Hausner's index
This was calculated as the ratio of tapped density to bulk density of the samples.

Compressibility index
This was calculated using the equation:
Compressibility = (Tapped density − bulk density)/Tapped density × 100.

Differential Scanning Calorimetry (DSC) Analysis
Thermal properties of MIG powder were characterized using a Shimadzu DSC-60, Shimadzu Limited Tokyo, Japan. Nitrogen, at the rate of 20 ml/min, was used as purge gas; 2 mg of powdered material were sealed in aluminium pan and heated from 30°C up to 400°C at the rate of 10°C/min, followed by a cooling cycle back to 30°C at the same rate.

Fourier Transform Infra Red (FT-IR) Analysis
The FT-IR spectrum of the sample was recorded in an IR spectrometer (FT–IR: 8101 M, Shimadzu, Japan), using potassium bromide (KBr) discs prepared from powdered samples mixed with dry KBr in the ratio 1:200. Triplicate measurements were made, and the spectrum with the clearest identifiable peaks was chosen.

Phytochemical Examination[23]
Preliminary tests were performed to confirm the nature of gum obtained. The chemical tests that were conducted are: Ruthenium red test, Molisch test, test for reducing sugars and Ninhydrin test.

Drug-excipient compatibility studies
This study has been done to check whether there is any compatibility related problems are associated with drug and the excipients used for the formulation of gels. The drug and excipients must be compatible with one another to produce a product that is stable, efficacious, attractive, and easy to administer and safe. If the excipients are new and not been used in formulations containing the active substance, the compatibility studies are of paramount importance. Thermal analysis, H.P.T.L.C, FTIR, can be used to investigate and predict any physicochemical interactions between components in a formulation and can therefore be applied to the selection of suitable chemically compatible excipients.

IR Spectroscopy

The IR spectral analysis of a drug and other excipients were taken using Press pellet technique (using KBr). The IR spectra's were determined by using 1601 PC Shimadzu UV Spectrophotometer.

DSC Studies

Differential Scanning Calorimetry was performed on a Shimadzu DSC-60, Shimadzu Limited Japan. A 1:1 ratio of drug and excipient was weighed into aluminum crucible. And sample was analyzed by heating at a scanning rate of 20^0C over a temperature range 20^0-$300^{0.}$

HPTLC Studies
Drug and Excipients were subjected to HPTLC (CAMAG-HPTLC system, Switzerland). RF values of pure drug and drug with different Excipients were calculated.

Preparation of gels
Gels were prepared by using different concentrations of mucilage, drug, methyl paraben (preservative) and glycerin (plasticizer), as shown in Table 1 and stored in cool place until further use.

TABLE 1: Composition of Aceclofenac Gel formulation

Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**
MIG*(%)	2.0	2.5	3.0	3.5	4.0	4.5	5.0	5.5
Aceclofenac (%)	1	1	1	1	1	1	1	1
Glycerin (%)	10	10	10	10	10	10	10	10
Methyl paraben (%)	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Purified water q.s. to (g)	10	10	10	10	10	10	10	10

MIG* *Mangifera indica* gum

Evaluation of prepared gels
The prepared gels were evaluated for various evaluation parameters which includes;

***In vitro* diffusion profile**
Release of aceclofenac from various gel formulations (B5, B6, B7 and the commercial preparation Acent®, Intas, Ahmedabad) were studied employing the permeation apparatus as described Fites *et al.* A glass cylinder with both ends open, 10 cm height and 3.7 cm outer diameter was used as a permeation cell. A cellophane membrane (0.8 µm pore size, cut to suitable size, boiled in distilled water for 1 h and soaked in phosphate buffer of pH 7.4) was fixed to one end of the cylinder by adhesive tape. One gram of the prepared gel was taken in the cell (donor compartment) and the cell was immersed in a beaker containing 100 ml of phosphate buffer of pH 7.4 (receptor compartment). The cell was immersed in to a depth of 1 cm below the surface of buffer, which was agitated by a magnetic stirrer and the temperature was maintained at 37° ± 1° throughout the experiment. Aliquots were withdrawn from the receptor compartment periodically (0.5, 1, 1.5 and 2 h). After each withdrawal, the volume of liquid in the receptor compartment was replaced by phosphate buffer of pH 7.4. The drug concentration was determined spectrophotometrically (UV-1700, Shimadzu, Japan) at 274 nm.

Skin irritation study
Guinea pigs (400-500 g) of either sex were used for testing of skin irritation. The animals were maintained on standard animal feed and had free access to water. The animals were kept under standard conditions. Hair was shaved from back of guinea pigs and area of 4 cm^2 was marked on both the sides, one side served as control while the other side was test. Gel was applied (500 mg/guinea pig) twice a day for 7 d and the site was observed for any sensitivity and the reaction if any, was graded as 0, 1, 2, 3 for no reaction, slight patchy erythema, slight but cofluent or moderate but patchy erythema and severe erythema with or without edema, respectively.

Consistency
The measurement of consistency of the prepared gels was done by dropping a cone attached to a holding rod from a fix distance of 10cm in such way that it should fall on the centre of the glass cup filled with the gel. The penetration by the cone was measured from the surface of the gel to the tip of the cone inside the gel. The distance traveled by cone was noted down after 10sec[24].

Homogeneity
All developed gels were tested for homogeneity by visual inspection after the gels have been set in the container. They were tested for their appearance and presence of any aggregates.

pH
The pH of the various gel formulations was determined by using digital pH meter.

Spreadability
It was determined by wooden block and glass slide apparatus. Weights about 20g were added to the pan and the time was noted for upper slide (movable) to separate completely from the fixed slides[25].
Spreadability was then calculated by using the formula:
S = M.L/ T
Where,
S = Spreadability
M = Weight tide to upper slide
L = Length of glass slide
T = Time taken to separate the slide completely from each other.

Drug content
A specific quantity (100mg) of developed gel and marketed gel were taken and dissolved in 100ml of phosphate buffer of pH 7.4. The volumetric flask containing gel solution was shaken for 2hr on mechanical shaker in order to get complete solubility of drug. This solution was filtered and estimated spectrophotometrically (UV-1700, Shimadzu, Japan) at 274 nm.
using phosphate buffer(pH 7.4) as blank[26].

Viscosity
Viscosity was determined using Brookfield synchronic viscometer with helipath stand at room temperature with a shear rate of 5 rpm for 5 min.

Accelerated stability studies[27]
All the selected formulations were subjected to a stability testing for three months as per ICH norms at a temperature of 40º ± 2ºC in stability chambers (Lab-Care, India). All selected formulations were analyzed for the change in appearance, pH or drug content and also physical stability and synersis (spontaneous contraction of gel exuding some of the fluid medium).

***In vitro* anti-inflammatory activity**
The *in vitro* anti-inflammatory activity of the gel formulation was performed using carrageenan induced rat hind paw edema model. The Wistar albino male rats weighing 150 - 210 g were fasted overnight, but water was allowed *ad libitum*. The animals were divided into three groups of six animals each. Group I (control) received placebo gel, group II received 1.2 mg/mL of aceclofenac suspension in water and the group III received 1.2 mg/kg equivalent to aceclofenac in gel formulation. Immediately after drug administration 0.05 mL of 1% w/w solution of carrageenan was injected into the planter surface of the hind paw. The hind paw volume was measured at

different time intervals for 6 h after carrageenan treatment using a plethysmograph. The percent inhibition in hind paw edema volume was calculated using the following formula and compared with those recorded for control group.

Anti-inflammatory activity (%) = (1-A/B) × 100

Where **A** is the change in paw volume in the treated group and **B** is the change in paw volume in the control group.

Results and Discussion

The fact for increase in importance of natural plant based material is that plant resources are renewable and if cultivated or harvested in a sustainable manner, they can provide a constant supply of raw materials. However, substances from plant origin also pose several potential challenges such as being synthesized in small quantities and in mixtures that are structurally complex, which may differ according to the location of the plants as well as other variables such as the season. This may result in a slow and expensive isolation and purification process. Another issue that has become increasingly important is that of intellectual property rights.

Physicochemical characterization of *Mangifera indica* gum

The average yield of dried gum obtained from *Mangifera indica* tree was 35% w/w. The gum obtained was an off white to cream yellow color powder, and the viscosity of its 1% aqueous dispersion was 600 cP. The powder was slightly soluble in water and practically insoluble in ether, acetone, chloroform, methanol and ethanol.

The swelling characteristic of MIG was studied in different media; 0.1N hydrochloric acid, phosphate buffer (pH 7.4) and water. The swelling was highest in water (20) followed by 0.1N HCl pH (15) and least in phosphate buffer (10). Generally, the results show that MIG has high swelling index suggesting that the gum may perform well as binder/disintegrant/matrixing agent. The gum is a pH responsive polymer, it is therefore a "smart polymer," and may find application in controlled release dosage formulations. The moisture content of MIG was low (1.5%), suggesting its suitability in formulations containing moisture sensitive drugs. The total ash, water soluble ash and acid insoluble ash value of MIG was found to be 2.23, 1.3 and 0.4%w/w respectively. Ash values reflect the level of adulteration or handling of the drug. The bulk and tapped densities give an insight on the packing and arrangement of the particles and the compaction profile of a material. The compressibility index, Hausner ratio and angle of repose of MIG were 16.33%, 0.15 and 22.35° respectively, implying that the MIG has a good flow with moderate compressibility. The loss on drying, ash value and microbial count were well within official limits. The gum obtained from *Mangifera indica* tree was subjected to physicochemical characteristics the results of which are summarized in table 2.

Phytochemical screening of *Mangifera indica* gum

Phytochemical tests carried out on MIG confirmed the absence of alkaloids, glycosides and tannins. On treatment of mucilage with ruthenium red, it showed red colour confirming the obtained product as mucilage. A violet ring was formed at the junction of two liquids on reaction with Molisch's reagent indicating the presence of carbohydrates. Mucilage could not reduce Fehling's solution, so the sugars present were non reducing sugars. It reduced Fehling's solution after hydrolysis for 1h with concentrated sulfuric acid under reflux. Mucilage on treating with ninhydrin reagent does not give purple colouration indicating the absence of amino acids. The results of phytochemical screening of MIG are summarized in table 3.

Table 2: Physicochemical characterization of *Mangifera indica* gum

Parameters	**Observation**
Solubility	Slightly soluble in water, practically insoluble in alcohol, chloroform and acetone. Forms thick gel in water.
pH (1% w/v solution)	6.5
Loss on drying	1.5%
Ash value	2.23%
Water soluble ash	1.3%
Acid insoluble ash	0.4%
Sulphated ash	1.03%
Test for foreign matter	Less than 0.1%
Test for arsenic	Less than 1ppm
Swelling ratio	
In water	20.0
In 0.1 N HCl	15.0
In phosphate Buffer 7.4	10.0
True density	1.7g/dl
Bulk density	0.48 g/cc
Tapped density	0.56 g/cc
Compressibity index	16.33%
Hausner ratio	0.15
Angle of repose	22.35
State	Amorphous powder
Odor	No characteristic odor
Taste	Tasteless
Color	Off white- cream yellow color
Total bacterial count	
E.coli	Absent
Salmonella typhi	Absent
S.aureus	Absent
Yield (%)	35
Viscosity (1%)	600 centipoise

Table 3: Phytochemical screening of *Mangifera indica* gum

	Tests	Observation
1.	Test for Carbohydrates(Molisch's test)	+
2.	Test for Tannins(Ferric chloride test)	-
3.	Test for proteins (Ninhydrin test)	-
4.	Test for alkaloids (Wagner's test)	-
5.	Test for glycosides(Keller – Killaini test)	-
6.	Test for mucilage (Ruthenium red test)	+
7.	Test for flavonoids (Shinoda test)	-
8.	Test for reducing sugar (Felhing's test)	-
9.	Mounted in 95% alcohol	Transparent angular masses under microscope
10.	Mounting in the iodine	No blue colored particles (starch absent)
11.	Test with cupric –tartaric solution	Red precipitate is produced
12.	Warming with 5M sodium hydroxide	A brown color is produced
13.	Test for chlorides(silver nitrate test)	-
14.	Test for sulphates (barium chloride test)	-

Toxicity study of MIG

To determine the safety level of extracted MIG, acute and chronic toxicity studies were carried out. In acute toxicity study no mortality was observed even at 5000mg/kg of MIG on oral administration and all animals were found to be normal during and at the end of the observation period of three days. Food and water consumption also did not differ significantly and there was no change in general behavior or other physiological activities of the animals in both control and treated groups. To assess the suitability of MIG for the oral delivery we have recorded the body weight profile for the animals during the chronic toxicity studies at regular intervals of 10 days. It was found that the body weight of both control and treatment group and the rate of increase in body weight were comparable. Hence, it could be inferred that chronic administration of the gum might not influence either the food intake or growth. Biochemical and hematological parameters were determined at the

end of 30 days of continuous administration of MIG suspension and the biochemical and hematological parameters were found to be comparable to that of normal mice. The results are shown in table 4 and 5 respectively. Histological examination of the main organs like liver, kidney, heart and brain were carried out at the end of 30days of chronic toxicity study. From this study it was revealed that there was no sign of pathological changes in both control and in treatment group.

Table 4: Results of biochemical parameters in rats treated with MIG

Treatment	ALP (U/L)	ACP (U/L)	AST (U/L)	ALT (U/L)	Urea (U/L)	Creatinine (U/L)
Control (0.5%CMC)***	65±4.15*	29±4.25	72±2.34	56±1.25	51±2.10	0.4±0.22
Treatment (MIG)**** 500 mg/kg)	68±4.38**	27±2.02	69±4.10	58±2.87	48±1.65	0.3±0.21

*Data represents as the mean ±SD of 6 animals; **Data represents as the mean ±SD of 16 animals; ***CMC; Carboxy methyl cellulose; **** MIG; *mangifera indica* gum

Table 5: Results of Hematological changes observed in rats during and after treatment of MIG for 30 days

Treatment	RBC (10^6/mm^3)	WBC (10^3/mm^3)	Hb(g/dl)	N	L	E
Control(0.5% CMC)	4.3±0.05*	7100±0.10	13.58±0.21	8±0.52	85± 0.17	0±0.00
Test(MIG) 500 mg/kg)	4.1±0.07**	6850±0.13	14.12± 0.35	12±0.41	90± 0.21	1± 0.22

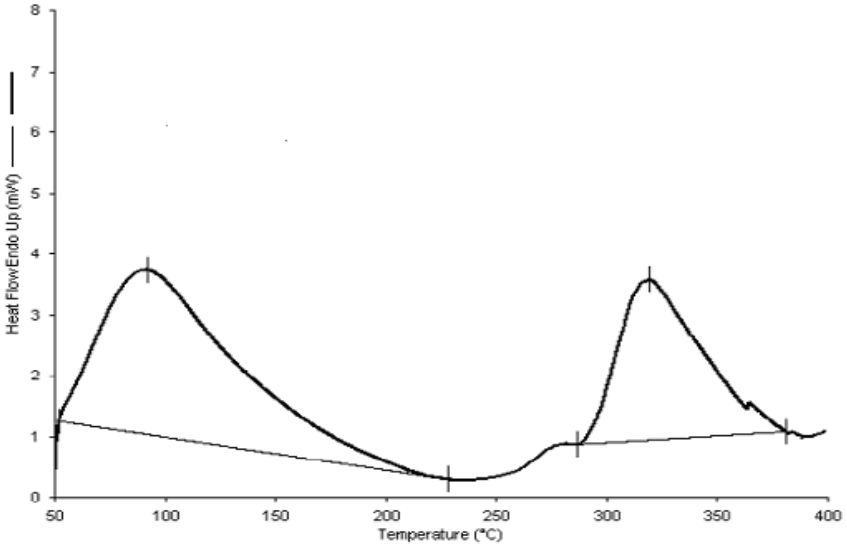
*Data represents as the mean ±SD of 6 animals; **Data represents as the mean ±SD of 16 animals

Characterization of MIG

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) was used to measure the occurrence of exothermal or endothermal changes with increase in temperature. DSC, because of its sensitivity and accuracy, has been extensively used to study the phase transitions of polymers. The thermogram for MIG is shown in Figure1. It shows that the gum has both amorphous and crystalline portions. Glass transition (Tg) temperature occurred at 94°C while a melting peak was observed at about 320°C.

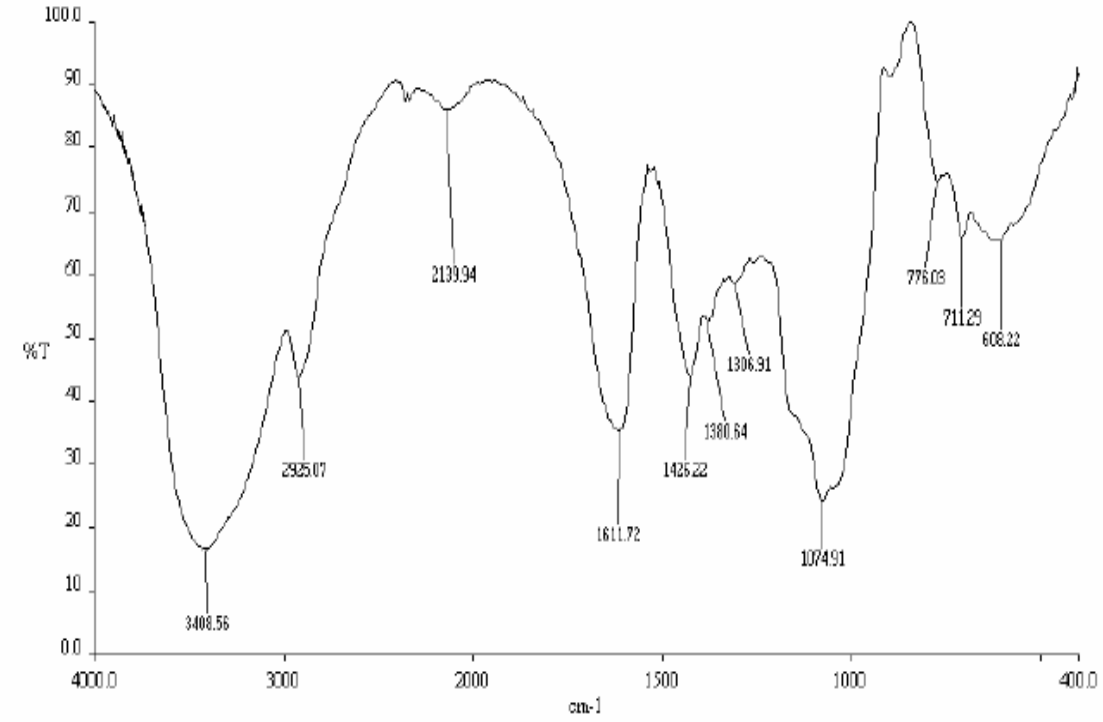
Fig.1: Differential scanning calorimetry curve of *mangifera indica* gum Powder



Fourier Transform Infra Red (FT-IR)

The IR spectrum of MIG is shown in Figure 2. The finger print region of the spectrum consists of two characteristic peaks between 700 and 1316 per cm, attributed to the C-O bond stretching. The band at 1604 per cm was assigned to the O-H bending of water. There are absorptions (weak) in the 1730 per cm area that indicate carbonyls. The absence of significant aromatic stretches in the 1660-1690 per cm region and the weakness of the stretches, imply that there is a modest amount of peptidic cross linking by amide bond formation. The sharp band at 2939 per cm is characteristic of methyl C-H stretching associated with aromatic rings. The broad band at 3286 cm^{-1} is due to the hydrogen-bonding that contributes to the complex irrational stretches associated with free inter and intra-molecular bound hydroxyl groups which make up the gross structure of carbohydrates.

Fig.2: FTIR spectrum of *mangifera indica* gum powder



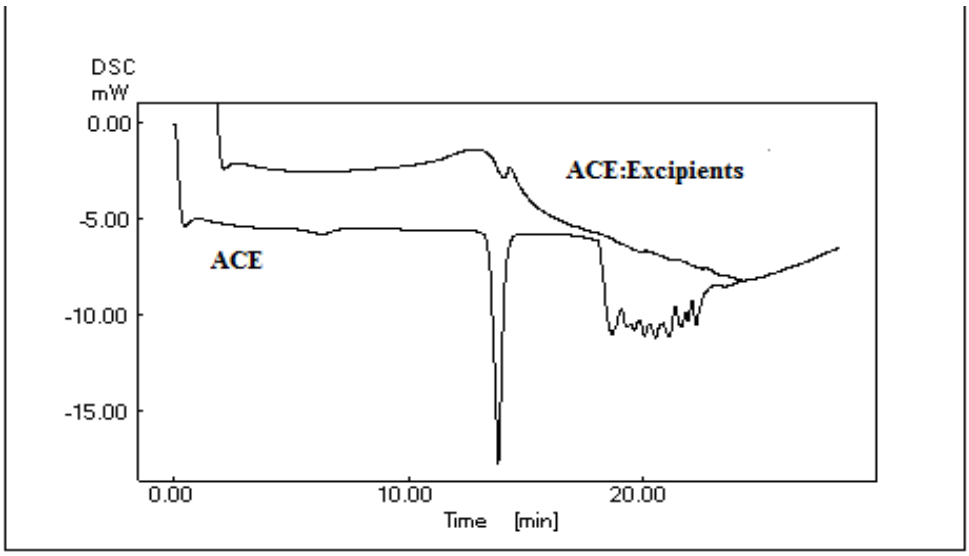
Characterization of drug and excipients

Differential Scanning Calorimetry (DSC)

DSC is useful in the investigation of solid-state interactions. The DSC analysis of pure aceclofenac showed a sharp endothermic peak at 156.54°C corresponding to its melting point.

The thermograms were generated for pure drug and drug excipient mixtures. The DSC analysis of physical mixture of the drug and excipients revealed negligible change in the melting point of aceclofenac in the presence of other excipients (152.91°C for the mixture of aceclofenac and mucilage). The thermograms are shown in Figure 3.

Fig.3: DSC Thermograms of aceclofenac alone and its physical mixtures



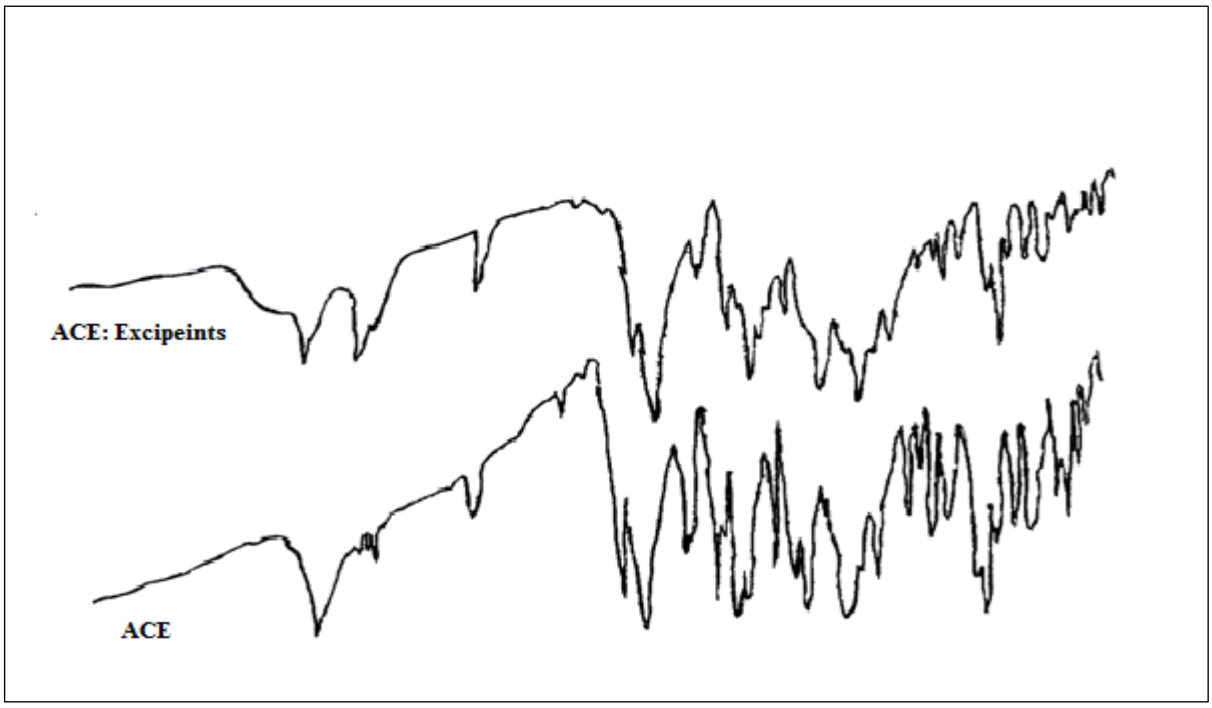
*ACE: Aceclofenac

Fourier transform infra red spectroscopy (FTIR)

The IR spectral analysis of aceclofenac alone showed that, the principle peaks were observed at wave numbers of 3276, 1770 and 3317 cm^{-1}. Confirming the purity of the drug as per the established standards. In the IR spectra of the physical mixture of the aceclofenac and excipients, the major peaks of aceclofenac were 3276, 1770 and 3317 cm^{-1} wave numbers. However no additional peaks were observed in physical mixture of the aceclofenac and excipients. IR spectra are shown in Figure 4.

Fig.4: IR Spectrum of aceclofenac alone and its physical mixtures

*ACE: Aceclofenac



HPTLC analysis

In HPTLC analysis the R_f value of pure aceclofenac was found to be 0.95. In presence of other excipients the R_f value of the drug was unchanged and found to be 0.95. DSC, HPTLC and FTIR results revealed that there is no interaction between the drug and the excipients used in the formulation.

Results of Evaluation parameters of aceclofenac gel

The gelling concentration of the gum was found to lie between 4.0 and 5.5% w/v but better gel characteristics were observed at the concentration of 4.5%. The pH of the gum was below 6.7, which is ideal for topical application. Eight batches of gel were prepared corresponding to 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 and 5.5 % w/w of MIG, 1% w/w of aceclofenac sodium, 0.2% w/w methyl paraben as preservative and 10% w/w glycerin as plasticizer. The pH values of those batches were determined. There was no significant difference in pH between pure gum solution and the different batches of gels formulated. Hence the gels were ideal for topical application. Among the prepared gels the batch containing 4.5% gum had opaque color without any characteristic odor and pH of 6.7. Therefore this was considered as ideal batch. The gels exhibited pseudo plastic flow (shear thinning) the viscosity was found to be ideal for topical application. The stability of the gel was determined 40º ± 2ºC. Precipitation or turbidity occurs in some of the batches (F1, F2, F3 and F4) gel containing aceclofenac sodium which could be due to the incompatibility in the system due to presence of glycerin or propylene glycol at accelerated temperature. Hence, these batches were

discarded and remaining batches (F5, F6 and F7) were considered for further study. The study revealed that the gel formulations containing 4.5, 5.0, and 5.5% w/w of gum were physically stable and synerisis was not observed where as other formulations showed synerisis. Hence these three formulations were considered for *in vitro* diffusion study along with a marketed formulation. Skin irritation study revealed no sensitivity reaction.

The pH values of all developed (F5, F6 and F7) and marketed gel was 6.7. The values of spreadability indicate that the gel is easily spreadable by small amount of shear. Spreadability of marketed gel was 6.0g.cm/sec while F6 was 6.5g.cm/sec, indicating spreadability of MIG (at a concentration of 4.5%) containing aceclofenac sodium gel was good as compared to the marketed gel. The consistency reflects the capacity of the gel, to get ejected in uniform and desired quantity when the tube is squeezed. Consistency in terms of distance travel by cone was 5.5mm of all developed batches as compared to 8 mm of marketed gel. Consistency is inversely proportional to the distance traveled by falling cone. Hence, the consistencies of gum (at a concentration of 4.5%) containing aceclofenac sodium gel were better as compared with marketed gel. All developed and marketed gel showed good homogeneity with absence of lumps. The developed preparations were much clear and opaque as compared to marketed gel. The skin irritation studies of developed gel were carried out on guinea pig and that confirmed the absence of any irritation on the applied surface. During the stability studies the appearance was clear and no significant variation in pH was observed. Considering the accelerated stability studies and physiochemical parameters, batch F6 was selected for *in vitro* permeability release studies as well as compared with the marketed gel. The results are tabulated in table 6 and 7.

Table 6: Results of evaluation parameters of selected batches of aceclofenac gel

Batch No	pH	Spreadability (g.cm/sec)	Consistency (60 sec)	Homogeneity	Skin irritation test	Drug content (%)	Physical Appearance
F5	6.72 ± 0.02	5.0	5.5	Homogeneous	Nil	99.98	White
F6	6.71 ± 0.02	6.5	5.5	Homogeneous	Nil	99.76	Opaque
F7	6.67 ± 0.02	6.0	5.5	Homogeneous	Nil	99.89	White
Marketed gel	6.70 ± 0.02	6.0	8.0	Homogeneous	Nil	99.88	Opaque

Table 7: Stability study of selected batches of aceclofenac gel.

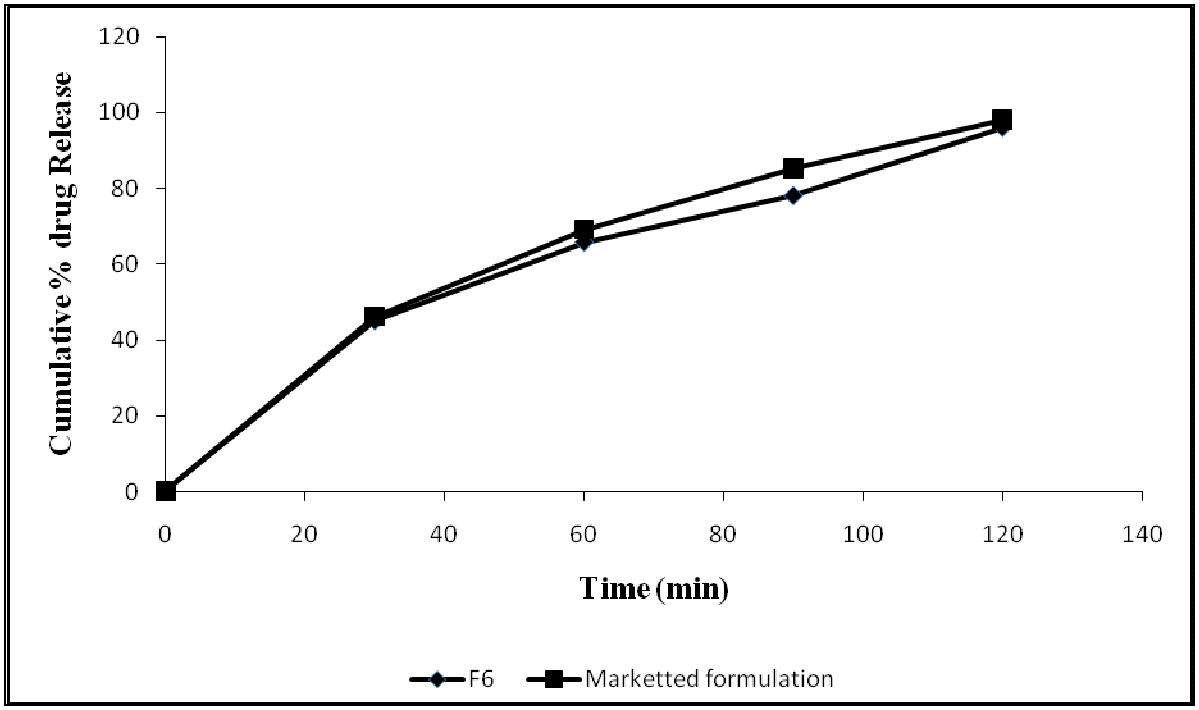
Batches	Months	pH	Appearance	Drug content	Consistency	Spreadability
F5	0	6.72	white	99.98	NC*	NC*
	1	6.73	white	99.92	NC	NC
	2	6.72	white	99.0	NSC	NSC
	3	6.71	white	98.5	NSC	NSC
F6	0	6.71	Opaque	99.76	NC	NC
	1	6.72	Opaque	99.65	NC	NC
	2	6.73	Opaque	99.0	NC	NC

	3	6.71	Opaque	98.5	NSC**	NSC**
F7	0	6.67	white	99. 89	NSC	NSC
	1	6.64	white	99.80	NSC	NSC
	2	6.62	white	99.10	NSC	NSC
	3	6.61	white	98.45	NC	NC
Marketed gel	0	6.7	Opaque	99.88	NC	NC
	1	6.5	Opaque	99.80	NC	NC
	2	6.4	Opaque	99.50	NSC	NSC
	3	6.4	Opaque	98.40	NSC	NSC

NC* = No change; NSC**= No significant change

In vitro Permeability study showed that permeation studies of F6 and marketed gel were comparable. The results are shown in figure 5.

Fig. 5: Comparison of *in vitro* release profile of optimized batch (F6) and marketed formulation



Results of antiinflammatory activity of optimized aceclofenac gel formulation

Figure 6 represents the change in edema volume after carrageenan treatment with aceclofenac oral suspension, aceclofenac gel and control gel. As shown in Table 8 and Figure 7, the maximum 52.18% inhibition of edema was observed with oral aceclofenac at 3 h after carrageenan treatment and maximum 44.43% inhibitions of edema was observed with aceclofenac gel formulation at 3 h after carrageenan treatment. It may be due to the initial slower release of drug from the gel

formulation. The better antiinflammatory activity found with the aceclofenac gel treatment may be accelerated for controlled drug release and protection of drug from first-pass hepatic metabolism which is encountered in the oral route.

It was observed that MIG (at a concentration of 4.5%) containing aceclofenac sodium (batch F6) produced better spreadability and consistency as compared to marketed aceclofenac sodium gel. The developed F6 gel showed good homogeneity, no skin irritation, good stability and *in vitro* permeability was comparable with marketed gel. The MIG forms water washable gel because of its water solubility and has wider prospects to be used as a topical drug delivery system.

Table 8: Results of Percent inhibitions of hind paw edema

Formulations	Percentage Inhibition (%)					
	1h	2h	3h	4h	5h	6h
Control	-	-	-	-	-	-
Aceclofenac oral	0.32	5.25	52.18	42.43	32.10	18.47
Aceclofenac gel	0.65	4.27	44.43	40.15	28.18	14.19

Fig.6: Change in edema volume with aceclofenac oral, placebo gel and aceclofenac gel after carrageenan treatment.

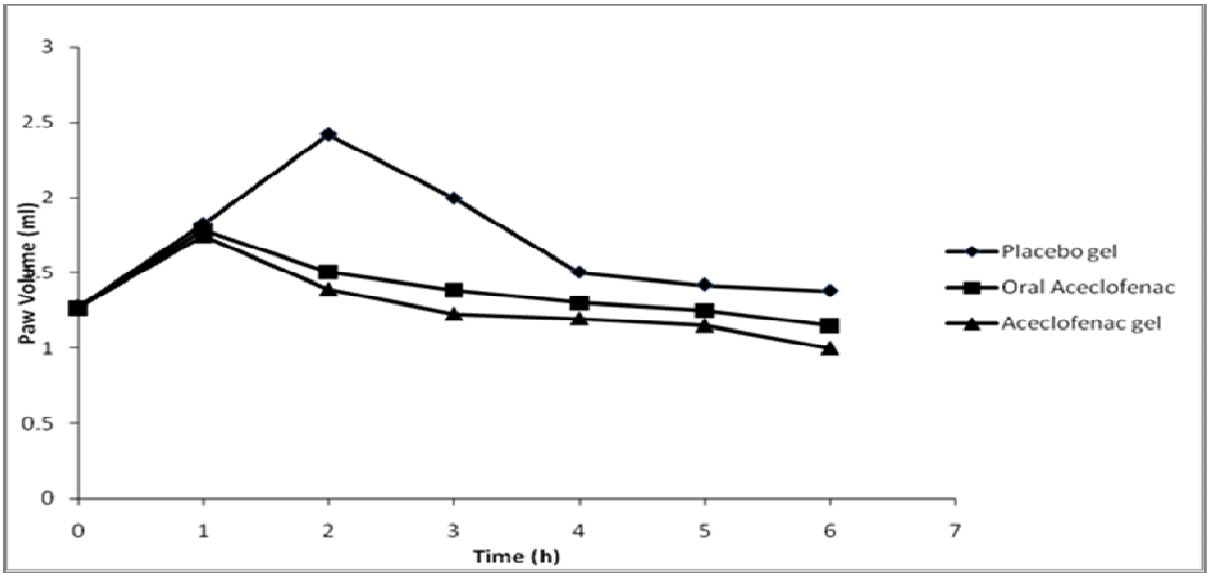
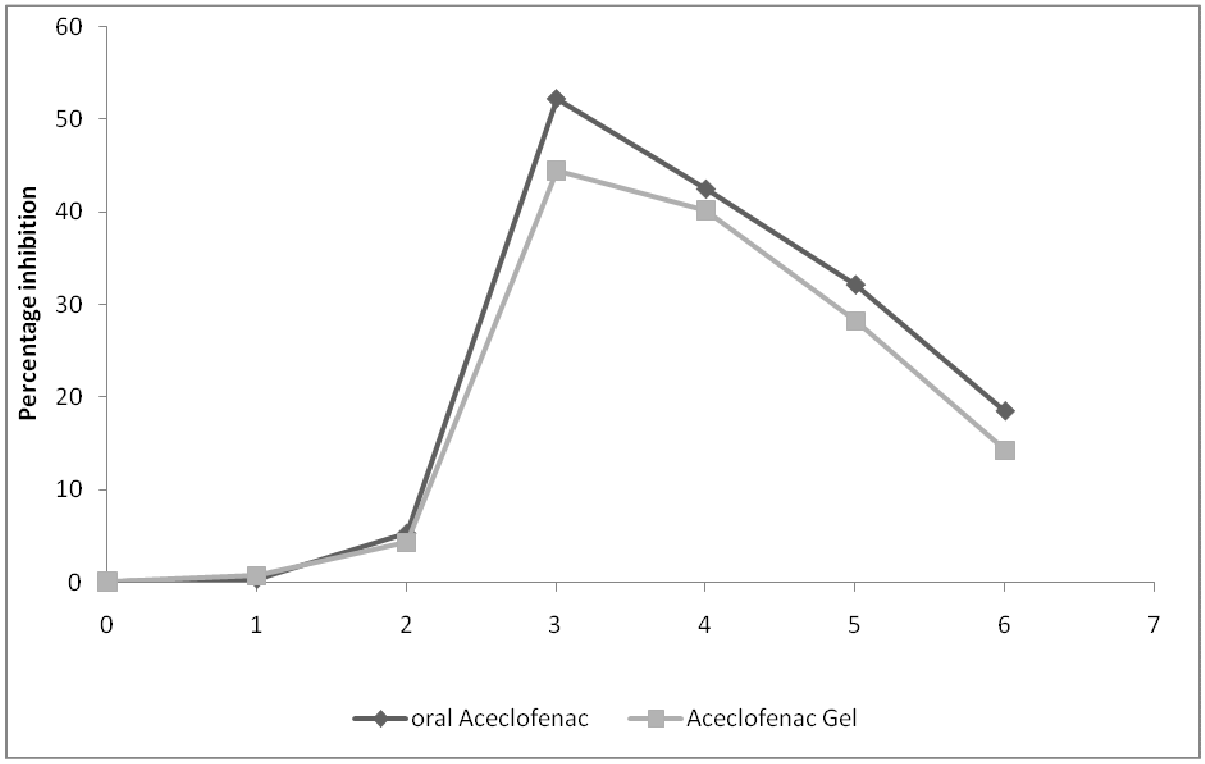


Fig. 7: Percent inhibitions of hind paw edema after oral administration of aceclofenac and application of aceclofenac gel.



Conclusion

From the present studies, it could be concluded that *Mangifera indica* gum can be used as gelling agent for the development of gel formulations, because of its good release profile, water-soluble nature, physical stability and good spreadability. The formulation F6 consisting of 4.5 % w/w *Mangifera indica* gum was found to be suitable for topical application based upon its physicochemical properties. The anti-inflammatory activity of this gel formulation in rat hind paw edema model reveals that aceclofenac was delivered to the inflammation site at a controlled level over a period of 3 h. The use of natural polymers for pharmaceutical applications is attractive because they are economical, readily available, non-toxic and capable of chemical modifications, potentially biodegradable and with few exceptions and also biocompatible. Majority of investigations on natural polymers in drug delivery systems center around polysaccharides. Natural gums can also be modified to have tailor-made products for drug delivery systems and thus can compete with the synthetic pharmaceutical excipients available in the market. They can be used as gelling agents in place of currently marketed synthetic gelling agents. Further studies will be worthy to establish the *Mangifera indica* gum as potent gelling agent.

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