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Evaluation of *Anacardium occidentale* gum as gelling agent in Aceclofenac Gel

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ABSTRACT: Mucilage extracted from *Anacardium occidentale* were subjected to toxicity studies for its safety and preformulation studies for its suitability as a gelling agent. The present study was undertaken with an objective to find out the gelling potentials of a natural gum obtained from plant *Anacardium occidentale*. The gum was extracted by using water as solvent and precipitated using acetone as non-solvent. Physico-chemical characteristics such as solubility, ash values, Precompression parameters, swelling index, loss on drying and pH were studied. In the present study eight batches of Aceclofenac gels were prepared with different concentration of mucilage (viz; 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 and 5.5). The gels were evaluated for drug content, viscosity determination, *in vitro* permeation (across dialysis membrane), skin irritation and stability tests. The gels prepared with 5.0% of mucilage 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. The gels were found to be stable with respect to viscosity, drug content and physical appearance at all temperature conditions for 3 months. Studies indicate that the extracted mucilage may be a good source as a pharmaceutical adjuvant specifically as a gelling agent.

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

INTRODUCTION

Excipients are the additives used to convert active pharmaceutical ingredients into pharmaceutical dosage form suitable for administration to patient[1]. New and improved excipients continue to be developed to meet the needs of conventional drug delivery systems. Plant products serve as an alternative to synthetic products because of local accessibility, environment friendly nature and lower prices compared to imported synthetic products. Herbs are non-polluting renewable resources for sustainable supplies of cheaper pharmaceutical products. Today, we have a number of plant-based pharmaceutical excipients. A number of researchers have explored the utility of plant-based materials as pharmaceutical excipients[2-8]. Majority of investigations on natural polymers in drug delivery systems are centered on polysaccharides and proteins, due to their ability to produce a wide range of materials and properties based on their molecular structures[9].

The gum in the present study is an exudate from the stem of the tree *Anacardium occidental*. The gum is initially off white in color but changes to reddish brown or yellowish brown on exposure. It is sparingly soluble in water but swells in contact with it giving a highly viscous solution. It is a polyuronide consisting of arabinose, galactose, rhamnose, and xylose. There are several reports about the successful use of hydrophilic plant polymers as tablet binders, emulsifiers, and gelling agents, suspending agents, stabilizers, and thickeners. When the gum mucilage is mixed with water, a protective soothing preparation results, which when applied externally will protect lesion or ulcer, from environmental contamination, infection, and sepsis.

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-Inflammatory Drug[10-13], which is chemically,(2-[2-[2-(2,6-dichlorophenyl)aminophenyl]acetyl]oxyacetic acid)[14-15]. Aceclofenac exhibited potent Anti-Inflammatory Analgesic activity and is widely prescribe for the treatment of osteoarthritis, rheumatoid arthritis, acute lumbago, and dental pain condition[16]. 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.

The objective of present work was to isolate gum from *Anacardium occidentale* Linn. plant and explore its use as a gelling agent.

MATERIALS AND METHODS

The gum was collected locally from the trees. Aceclofenac was obtained as gift sample from (Aristo Pharmaceuticals Ltd, Mumbai, India). All other materials, solvents, and reagents were of analytical grade.

Isolation of gum

The gum was collected from trees (injured site). It was dried, ground, and passed through sieve no 80. Dried gum (20 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° under vacuum.

Evaluation of toxicity

Toxicity studies were carried out according to the method of Knudsen and Curtis[17]. The animals used in the toxicity studies were sanctioned by the Institute animal Ethics Committee. The male albino rats of Wistar strain weighing 160-200 gm were divided into different groups comprising of six animals each. The control group received normal saline 20ml/kg i.p. The other groups received 500, 1000, 2000, 3000 and 4000 mg/kg of gum 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 48 hours. Since no mortality, no toxic manifestations were observed and behavioural pattern was unaffected. In chronic toxicity studies, 12 animals were used, divided in to two groups, 6 as control and 16 as test animals. In the test group a dose of 250 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, hematological parameters were studied in both the groups.

Investigation for color change

Samples of fresh gum containing 2 ml of 2% w/v gum with 1 ml of 0.1% ascorbic acid and 1 ml of 0.1% sodium bisulfite as antioxidant were prepared in duplicate. Both the samples were poured on Petri dish and exposed to sunlight as well as kept in dark along with controlled samples without any antioxidant for a period of 12 h. After 12 h they were observed for change in color.

Physicochemical properties of mucilage

The physicochemical properties such as solubility, ash values, pre-compression parameters, microbial load, swelling index, loss on drying were determined according to Indian Pharmacopoeial Procedures. The pH of the mucilage was determined using a digital pH meter (Elico, Hyderabad).

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.

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[18].

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[19].
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[20].

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[21]

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

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 Fig. 1.

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 Fig. 2.

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.

Stem exudates of *Anacardium occidental* yielded 55% of gum when acetone was used for precipitation of the gum. The investigation for color change revealed that the presence of antioxidant does not prevent color change, whereas change in color was found to be more intense with samples exposed to sunlight. The physicochemical properties of gum were determined and are shown in table 2.

To determine the safety level of the extracted *Anacardium occidental* mucilage, acute toxicity and chronic toxicity studies were carried out. In both toxicity study of the gum revealed no behavioral changes for first four hours and no mortality, no toxic syndromes were observed even at the dose level 4000mg/kg body weight after 24 hours, indicating the safety of the gum. To assess the suitability of gum for the oral delivery we have recorded the body weight profile for the animals during the chronic toxicities at regular intervals of 10 d. it was found that the body weight of both test and control and rate of increase were also comparable. Hence it is concluded that chronic administration of the gum might not influence either the food intake or growth. Hematological parameters that were determined at the end of 30 d of continuous administration were also found to be comparable to that of control rat. The effect of *Anacardium occidental* mucilage on hematological parameters is summarized in table3.

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 5.0%. 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 *Anacardium occidental* gum, 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 5 % gum had opaque color without any characteristic odor and pH of 6.7. Therefore this was considered as ideal batch. The gels exhibited psedoplastic 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 mucilage (at a concentration of 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 6mm of all developed batches as compared to 9 mm of marketed gel. Consistency is inversely proportional to the distance traveled by falling cone. Hence, the consistencies of mucilage (at a concentration of 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 4 and 5.

In vitro Permeability study showed that permeation studies of F6 and marketed gel were comparable. The results are shown in fig.3.

Figure 4 represents the change in edema volume after carrageenan treatment with aceclofenac oral suspension, aceclofenac gel and control gel. As shown in Table 6 and Figure 5, the maximum 42.36% inhibition of edema was observed with oral aceclofenac at 3 h after carrageenan treatment and maximum 37.73% 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 mucilage (at a concentration of 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 mucilage of *Anacardium occidentale* forms water washable gel because of its water solubility and has wider prospects to be used as a topical drug delivery system.

CONCLUSION

A Gel provides a successful approach in delivering combination products hence for the present study Gel system has designed to deliver the drug aceclofenac and also improve physical appearance of the gel for topical application using mucilage of *Anacardium occidentale*. The formulation F6 consisting of 5% w/w *Anacardium occidentale* 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. These results suggest the feasibility of the topical gel formulation of aceclofenac. As primary ingredients are cheap, biocompatible, biodegradable and easy to manufacture. They can be used as gelling agents in place of currently marketed synthetic gelling agents.

FUTURE PERSPECTIVES

The present investigation is a primary platform to indicate the suitability of *Anacardium occidentale* mucilage as a gelling agent. The work can further be extended for evaluation of its suitability as suspending agent, binding, emulsifying agent and other similar pharmaceutical applications considering the easy and ample availability of the plant. The work can go a long way to evaluate herbal pharmaceutical excipients.

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TABLE 1: Formulation of Aceclofenac Gel

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8
AOM*(%)	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

AOM* *Anacardium occidental* mucilage

Table 2: Physicochemical Properties of Mucilage

SL.No.	Parameters	Result
1.	Solubility	It is sparingly soluble in water forms viscous solution, insoluble in ethanol, methanol, acetone and ether.
2.	pH	5.65
3.	Loss on Drying	8%
4.	Swelling index	22
5.	Total ash	2%
6.	Acid insoluble ash	0.5%

7.	Water soluble ash	1.0%
8.	Test for foreign matter	Less than 0.1%
9.	Test for carbohydrates(Molisch's test)	+
10.	Test for tannins (Ferric chloride test)	-
11.	Test for proteins (Biuret test)	-
12.	Test for chlorides (Silver nitrate test)	-
13.	Test for sulphates(barium chloride test)	-
14.	description	Powder: off white granular powder
15.	Angle of repose	22
16.	Compressibility index	20%
17.	Test for mucilage(Ruthenium red test)	+
18.	Tapped density	0.76
19.	True density	1.5g/dl
20.	Bulk density	0.54
21.	Yield	55%
22.	Microbial load Bacteria (CFUs/g)	
	Fungi (CFUs/g)	85
		5

Table3. Hematological values of male rats receiving *Anacardium occidental* mucilage for 3 months.

SL.No.	Parameters	Control	Test
1.	Hematocrit (%)	49.59±1.91	51.13±2.77
2.	RBC ($\times 10^6$ cells/mm^3)	8.70±0.49	8.75±0.41
3.	Hemoglobin (g/dl)	16.72±0.86	16.56±0.67
4.	MCV (μm^3/red cell)	58.51±1.86	58.77±1.60
5.	MCH (pg/red cell)	19.23±0.51	19.67±0.63
6.	MCHC (g/dl RBC)	32.57±0.37	32.73±0.54
7.	WBC ($\times 10^3$ cells/mm^3)	2.12±0.46	2.15±0.55
8.	Platelet ($\times 10^3$ cells/mm^3)	922±106	942±79
9.	Neutrophil	18.12±3.81	18.39±4.58
10.	Eosinophil (%)	1.51±0.46	1.62±0.56

11.	Lymphocyte (%)	64.90±6.21	65.22±4.54
12.	Monocyte (%)	10.50±5.35	11.01±3.72
13.	Basophil (%)	3.41±1.27	4.03±1.53
14.	Platelet ($\times 10^3$ cells/mm^3)	956±120	959±95

Table 4. Values of evaluation parameters of developed gel and marketed gel

Batch No	pH	Spreadability (g.cm/sec)	Consistency (60 sec)	Homogeneity	Skin irritation test	Drug content (%)	Physical Appearance
F5	6.70 ± 0.03	6.0	6.0	Homogeneous	Nil	99.90	White
F6	6.71 ± 0.04	6.5	6.0	Homogeneous	Nil	99.94	Opaque
F7	6.69 ± 0.03	7.0	6.0	Homogeneous	Nil	99.98	White
Marketed gel	6.71 ± 0.05	6.0	9.0	Homogeneous	Nil	99.95	Opaque

Table 5. Stability study of various developed gel and marketed gel.

Batches	Months	pH	Appearance	Drug content	Consistency	Spreadability
F5	0	6.7	white	99.90	NC*	NC*
	1	6.8	white	99.00	NC	NC
	2	6.6	white	98.50	NSC	NSC
	3	6.7	white	97.00	NSC	NSC
F6	0	7.09	Opaque	99.94	NC	NC
	1	7.1	Opaque	98.00	NC	NC
	2	7.2	Opaque	97.5	NC	NC
	3	7.1	Opaque	97.00	NSC**	NSC**
F7	0	6.56	white	99.98	NSC	NSC
	1	6.6	white	98.80	NSC	NSC
	2	6.7	white	98.12	NSC	NSC
	3	6.6	white	97.45	NC	NC
Marketed gel	0	6.9	Opaque	99.95	NC	NC
	1	7.0	Opaque	98.60	NC	NC
	2	7.1	Opaque	98.50	NSC	NSC
	3	7.1	Opaque	97.40	NSC	NSC

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

Table 6: Percent inhibitions of hind paw edema

Formulations	Percentage Inhibition (%)					
	1h	**2h**	**3h**	**4h**	**5h**	**6h**
Control	-	-	-	-	-	-
Aceclofenac oral	0.23	4.21	42.36	38.38	20.30	19.01
Aceclofenac gel	0.55	2.35	37.73	30.65	13.44	12.11

Fig1. DSC Thermograms of aceclofenac alone and its physical mixtures

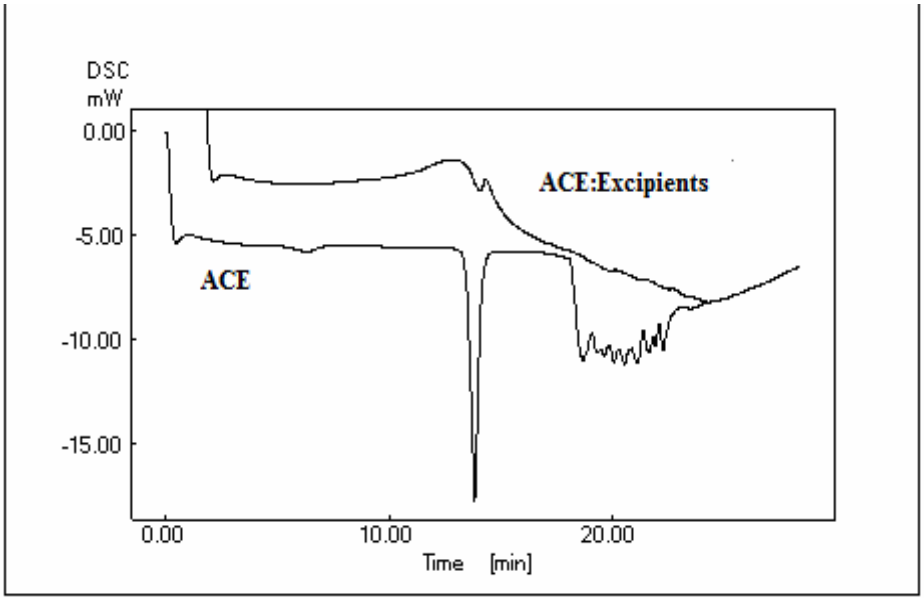


Fig2. IR Spectrum of aceclofenac alone and its physical mixtures

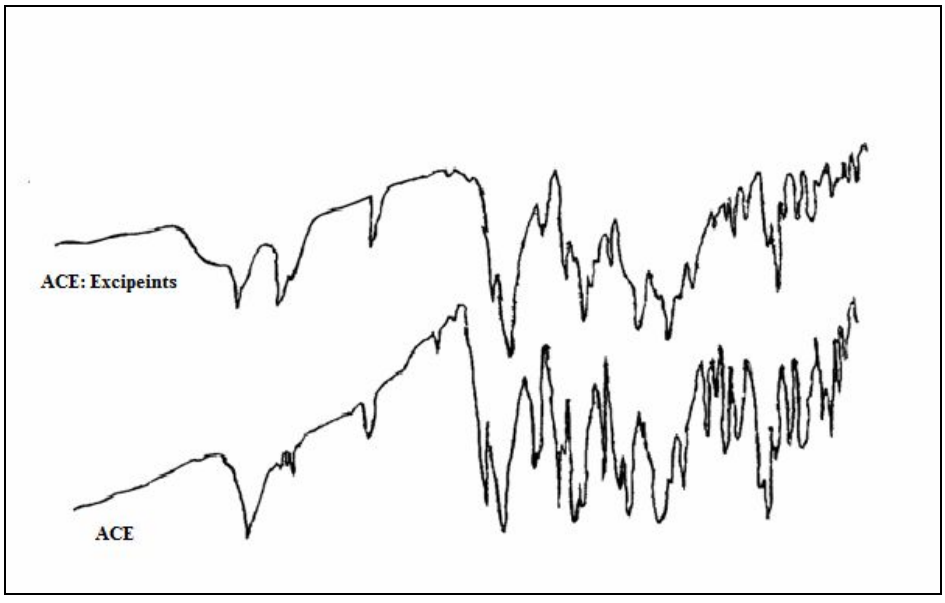


Fig 3. Drug permeability release profile of aceclofenac gel formulation.

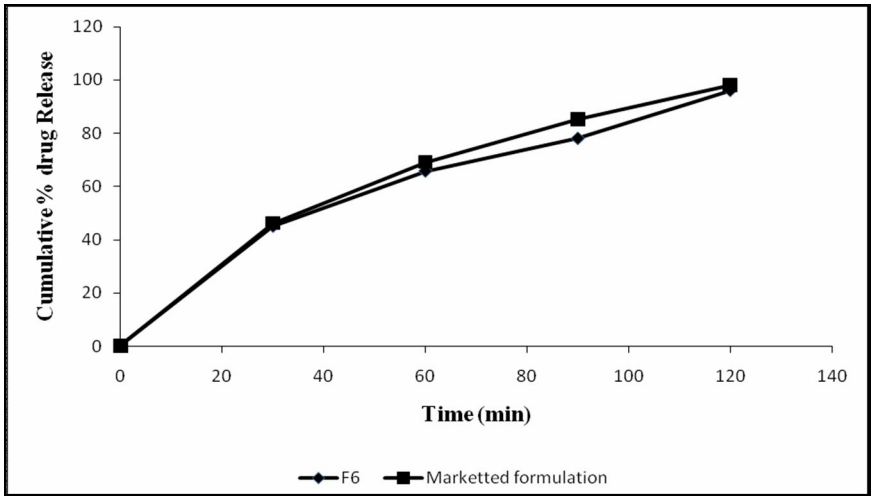


Fig4. Change in edema volume with aceclofenac oral, placebo gel and aceclofenac gel after carrageenan treatment.

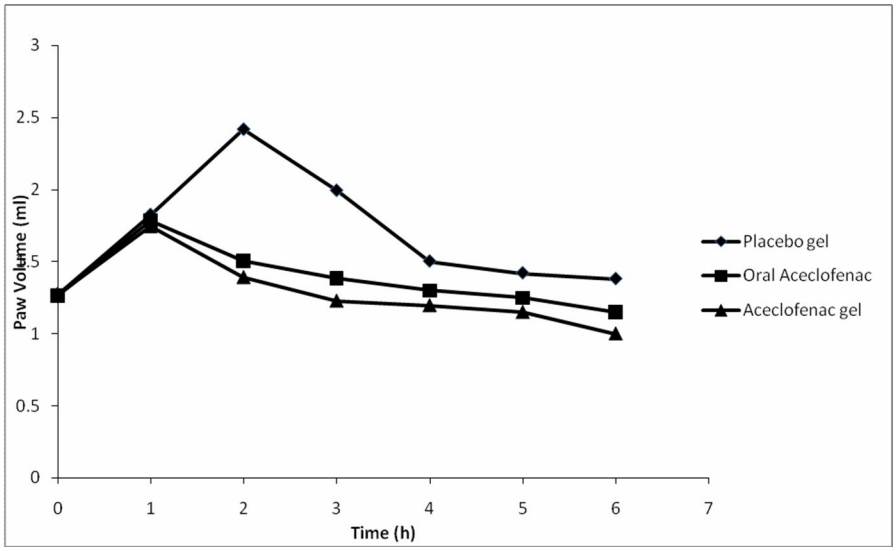
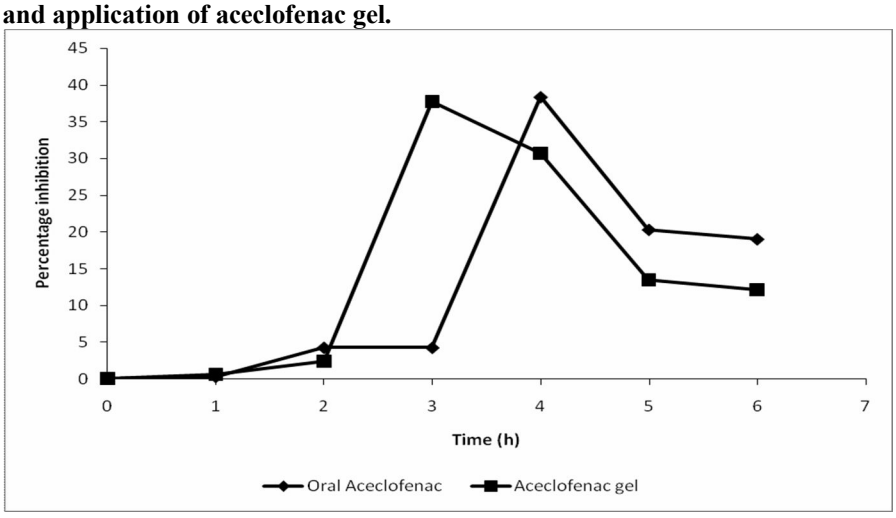


Figure 5. Percent inhibition of hind paw edema after oral administration of aceclofenac and application of aceclofenac gel.



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