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Preparation and Evaluation of Flurbiprofen Gel; Mucilage of *Cocculus hirsutus* Leaf Powder as Gel Base

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Abstract: The aim of present work is to check the suitability of *cocculus hirsutus* leaf powder as a gel base. Leaves of *Cocculus hirsutus* (Sisi Leaves, Family: *Menispermaceae*) was collected, dried and powdered. A solvent acetone was used for the removal of chlorophyll. Physical characteristics of this powder such as swelling index, pH and viscosity were studied. Flurbiprofen was used as a model drug for the formulation of gel. The various ingredients used for the preparation of gel are glycerin as plasticizer, benzyl alcohol as preservative and plant mucilage as gelling agent. The prepared gel and marketed available Flurbiprofen gels are evaluated for anti-inflammatory property by using carrageenan induced acute paw oedema in rats. The following parameters such as pH, stability, viscosity, skin irritation test and *In-vitro* diffusion test were done for both test and marketed gels. Both results were well co-related. It is observed that the quantity of Flurbiprofen released form prepared test gel and its anti-Inflammatory activity is more than that of marketed gel. It is concluded that the plant mucilage is well suited for the formulation of gel.
Keywords: Flurbiprofen, Gel base, Carrageenan, *Cocculus hirsutus*, Plasticizer, Acetone.

INTRODUCTION

The high cost of synthetic polymers and environmental pollution by chemical industry has made the scientists in developing countries to enter into an era, in which plant products serve as alternative to synthetic products because of their economy, local accessibility and environmental friendly nature.[1]

Today, we have a number of plant based pharmaceutical excipients such as starch, agar, alginates, acacia, cocoa butter, mucilage, celluloses…etc. These natural materials are used as diluents, binders, disintegrants in tablet, protective colloids in suspension, thickeners in oral liquid, gelling agents in gel and bases in suppository. Plant mucilage's, which provide high concentration of complex sugars and these, when mixed with water, a protective and soothing preparation results, which can be applied externally. In the present study, cocculus hirsutus leaves were selected for the formulation of external gel preparation because of its low cost and easy avaibility.[2]

Cocculus hirsutus leaves contain a high proportion of mucilage. The majority of the traditional uses of Sisi leaves can be attributed to the mucilage content only. This mucilage contains polysaccharides and a gelatinous type of material. This material is not absorbed in the G.I.T, and passes through the system undigested. *Cocculus hirsutus* leaves are used topically as emollient and demulcent. It has been non-toxic to human skin. Hence in the present study, it was planned to formulate Flurbiprofen gel using cocculus mucilage as a gelling agent and to study its characters.[3]

MATERIALS AND METHODS

The plant leaves were collected in November 2007 from Thallapaka and Rajampeta regions, Kadapa dist., Andhra Pradesh. The plant was authentified (Authentification No.1311) by Dr.C.Madhava Chetty, Head of Department of Botany, S.V.University, Tirupati. Flurbiprofen was obtained as gift sample from Seeko Laboratories, Vijayawada, Andhra Pradesh. All the ingredients used were of analytical grade only.

The fresh plant leaves washed under the running tap water, dried under the shade, and powdered by using hand grind mill and mixi. The resulted powder was passed through sieve no.20. The chlorophyll of this leaf powder was removed by using acetone as a solvent.

Accurately weighed 1 gram of leaf powder and 8.6ml of distilled water was transferred into china dish, stirred and kept a side for 15 minutes. The wetted leaf powder was squeezed through white muslin cloth into 100ml beaker and this mucilage was used as gel base. The formula for gel base is showed in table no.1. Accurately weighed quantities of Flurbiprofen, Glycerin and Benzyl alcohol and gel base were mixed in beaker. A transparent gel was formed after 20 minutes. All the quantities and uses of ingredients are showed in table No: 2.[4-6]

EVALUATION OF PREPARED GEL

The prepared gel was evaluated for drug content, pH, viscosity, swelling index, *in-vitro* diffusion profile, skin irritation test, stability and carrageenan induced acute paw oedema in rats.

DRUG CONTENT DETERMINATION

The amount of Flurbiprofen present in the prepared gel was analyzed by using U.V.Spectrophotometer. Accurately weighed quantity of prepared gel equivalent to 50 mg of Flurbiprofen was extracted with 50 ml of methanol, filtered and it was diluted to 200 ml. Further 5 ml of this filtrate was dilulted to 100 ml in volumetric flask with methanol. The absorbance of this solution was measured at 247 nm by using U.V. Spectrophotometer. The amount of drug present in the gel and it's percentage purity was calculated by using following formulas respectively.[7]

Amount of drug = Concentration X dilution factor X conversion factor

Percentage purity =

$$= \frac{\text{Amount of drug present in prepared gel}}{\text{Labeled claim}} \times 100$$

pH DETERMINATION

Two grams of prepared gel was dissolved in the 100 ml of phosphate buffer solution and pH of the resulted solution was studied by digital pH meter with glass electrode.[8]

VISCOSITY DETERMINATION

Viscosity was determined by Brookfield viscometer which is thermo stated at a temperature of 35 ± 1^0 C. The spindle no. and velocity used are 07 and 20 rpm respectively.[9]

SWELLING INDEX DETERMINATION

Accurately weighed 1 gram of *cocculus* leaf powder was added to 25 ml measuring cylinder and distilled water was added up to the mark. The leaf powder was allowed to swell for 4 hours. The initial and final volumes occupied by the leaf powder are found as 7.3 and 15.4 ml respectively.[10]

IN-VITRO DIFFUSION PROFILE

In-Vitro Release of Flurbiprofen from the prepared gel was compared with marketed available gel (Brugel, Acme Pharma, Solan, India) by 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 hr and soaked in phosphate buffer of pH 7.2) was fixed to one end of the cylinder by adhesive tape. Two grams of the prepared gel was taken into the cell (donor compartment) and the cell was immersed in a beaker containing 100 ml phosphate buffer pH 7.2 (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 $37^0 \pm 1^0$C, throughout the experiment. Aliquots were withdrawn from the receptor compartment periodically (0, 1, 2, 3, 4, 5, 6….8 hours). After each withdrawal of 0.1 ml, the same volume of liquid in the receptor compartment was replaced by phosphate buffer of pH 7.2. This 0.1 ml of the solvent was diluted to 10 ml with phosphate buffer. The drug concentration was determined spectrophotometrically at 247 nm. The same procedure was fallowed for the marketed available gel formulation also and compared the cumulative percentage of drug release from both prepared and marketed gel. The amount of drug release was calculated by using following formula.[11, 12]

Amount of drug release = Concentration X Dilution factor X Conversion factor

Cumulative % of drug release =

$$= \frac{\text{Amount of drug release}}{\text{Amount of drug loaded}} \times 100$$

SKIN IRRITATION TEST

Guinea pigs (400 – 500 grams) 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 days and the site was observed for any sensitivity and reaction. The sensitivity was graded as 0,1,2,3, for no reaction, slight patchy erythema, slight but patchy erythema and severe erythema with or without edema, respectively.[13]

STABILITY TEST

Stability testing was done by using freeze thaw cycling method, the temperature was altered for every 24 hours between 25^0C and -5^0C for five cycles and samples were observed for physical stability and synersis. (Spontaneous contraction of gel exuding some of the fluid medium) [14].

ANTI-INFLAMMATORY ACTIVITY STUDIES

Albino rats of wistar strains of either sex between 140 – 170 grams were selected for the studies. The animals were kept on standard diet and allowed free access to water. The animals were divided into four groups comprising four animals in each group as

Group 1 :- For control
Group 2 :- Gel base (without drug)
Group 3 :- Prepared test gel
Group 4 :- Marketed available gel

Anti-inflammatory activity was evaluated using carrageenan induced rat hind paw edema method of Winter at al. 50 mg of respective formulation were applied to the paw one hour before carrageenan injection (0.1 ml 1%) subcutaneously. Paw volume was measured by Plethesmograph at the end of each and every hour. Test was carried for a period of 4 hours and % inhibition of oedema was calculated by using following formula.[15 - 18].

% of Inhibition of oedema = [1 - VT/VC] X 100
VT = Mean inflammation of test group.
VC = Mean inflammation of control group.

RESULT AND DISCUSSION

The leaf powder of *cocculus hirsutus* yielded high % of mucilage. This mucilage used as a gelling agent. Cocculus leaf powder also having the chlorophyll, this chlorophyll was removed by using acetone as a solvent. Composition of the prepared gel was mentioned in the table N0:- 1 and 2. The physicochemical properties of mucilage was determined and are shown in table:- 03. The pH of the prepared gel found to be 6.4, which is ideal for topical application. The gel exhibited psedoplastic flow (shear thinning) the viscosity was found to be ideal for topical application. The pharmacodynamic evaluation of the prepared flurbiprofen gel and marketed available gel values were showed on the table No:- 05.

In vitro diffusion profile:-

The in vitro diffusion profile of the prepared flurbiprofen gel and marketed available gel was compared and results were showed on the table no:- 04. The gel prepared with mucilage showed a maximum release of 87 % over a period of 8 hr, when compared with the marketed gel showed a maximum release of 85 % . The release profile of prepared and marketed available gels comparison was showed on the figure no:-1.

Stability studies:-

The stability of the gel was determined by freeze-thaw cycling method. The study revealed that the gel formulation was physically stable and no syneresis was observed.

CONCLUSION

Cocculus hirsutus leaves were collected, dried and powdered. The chlorophyll of the leaf powder was removed by using acetone. The Swelling index of this powder is determined. Flurbiprofen gel is prepared by using this plant mucilage as gel base. The various ingredients used for the preparation of gel are glycerin, benzyl alcohol.

The prepared and marketed available Flurbiprofen gels are evaluated for all the parameters such as pH, stability, viscosity, skin irritation, drug content, anti inflammatory activity test and *in-vitro* diffusion test. Both results were well co-related. From the results, it has been observed that, the amount of drug released from the prepared gel is more than that of marketed gel. It can be concluded that this prepared test gel is more efficient than marketed gel.

TABLE: - 1. FORMULA FOR PREPARATION OF GEL BASE.

S.NO	Ingredients	Use	Qty of Gel base For 30grams	Qty of Gel base For 5 grams
1	*Cocculus hirsutus* leaf powder	Gel base	6gram	1grams
2	Water	vehicle	51.6ml	8.6 ml

TABLE: – 2. FORMULA FOR PREPARATION OF GEL

S.no	Ingredients	Use	Standard formula For 25 grams
1	Flurbiprofen	Medicament	1.25grams
2	Gel base	Gel base	22.15 grams
3	Glycerin	Plasticizer	2.1 grams
4	Benzyl alcohol	Preservative	0.5grams

TABLE No:- 3, PHYSICAL CHARECTERISTICS OF PREPARED TEST GEL

S.no	Test	Result
1	pH	6.4
2	Swelling index	8.1
3	Viscosity	65,000–80,000 m poise
4	Stability	Good and No synersis
5	Skin irritation	0
6	Percentage purity	96%

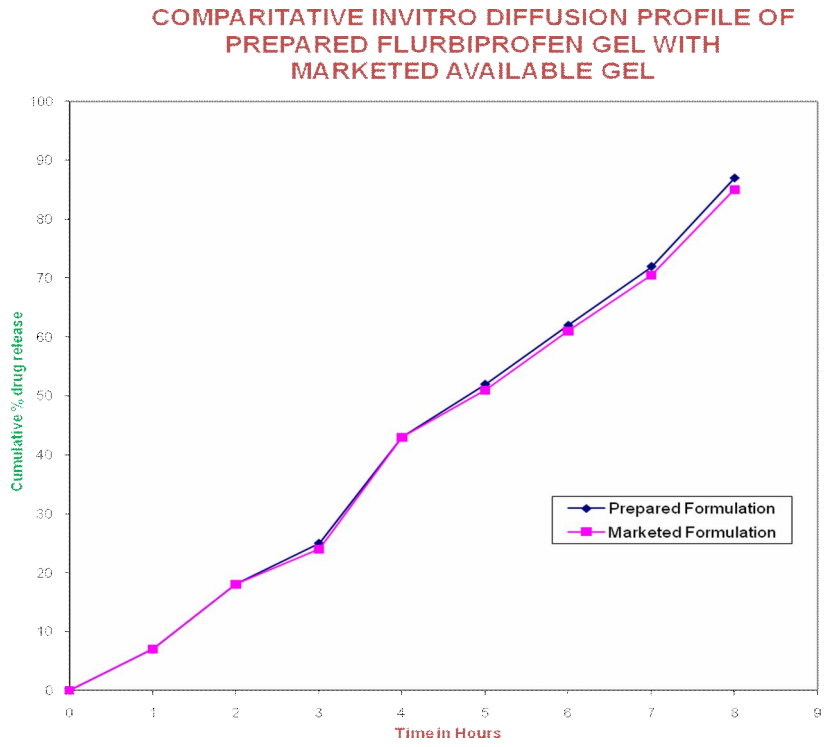
TABLE - 4. INVITRO DIFFUSION TEST

S.No	Time In Hours	Prepared gel formulation				Marketed gel formulation			
		Absorbance	Concentration in (µg/ml)	Amount of drug released In mg	Cumulative % of drug released	Absorbance	Concentration in (µg/ml)	Amount of drug released In mg	Cumulative % of Drug released
1	1hr	0.055	0.7	7	7	0.07	0.7	7	7
2	2hr	0.151	1.8	18	18	0.15	1.8	18	18
3	3hr	0.22	2.5	25	25	0.21	2.4	24	24
4	4hr	0.376	4.3	43	43	0.375	4.3	43	43
5	5hr	0.469	5.2	52	52	0.46	5.1	51	51
6	6hr	0.561	6.2	62	62	0.55	6.1	61	61
7	7hr	0.651	7.2	72	72	0.651	7.2	72	72
8	8hr	0.781	8.7	87	87	0.75	8.5	85	85

TABLE:- 5. PHARMACODYNAMIC STUDY

Group No	Treatment	Mean volume in ml				% of inhibition			
		1 hr	2 hr	3 hr	4 hr	1 hr	2 hr	3 hr	4 hr
1	Control	0.248 ± 0.013	0.681 ± 0.023	0.722 ± 0.013	0.762 ± 0.012	-	-	-	-
2	Gel base (without Drug)	0.248 ± 0.013	0.681 ± 0.023	0.722 ± 0.013	0.762 ± 0.012	0	0	0	0
3	Prepared Gel (Flurbiprofen)	0.166 ± 0.013	0.348 ± 0.012	0.242 ± 0.013	0.132 ± 0.012	33	48.8	66.48	82.6
4	Standard Marketed Available gel	0.169 ± 0.007	0.352 ± 0.010	0.248 ± 0.012	0.138 ± 0.013	31.8	48.3	65.5	81.8

GRAPH:1:



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