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ISSN: 0975-7538
Research Article

Development and evaluation of herbal gel formulation of *Magnifera indica* linn extract

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ABSTRACT

The aim of the present study is to develop topical gel formulation containing ethanol:water (80:20) dry extract of *Mangifera indica* L. and to evaluate its properties with respect to its quality, effectiveness against inflammation and stability. Different gel formulations containing ethanol:water dry extract of *Mangifera indica* Linn were prepared using various concentrations of Carbapol 934 P and HPMC separately. The formulation were evaluated and its anti-inflammatory activities compared with standard formulations were also subjected to intermediate and accelerated stability studies as per ICH guideline. The Gel were found to be non-newtonian; viscoelastic with equivalent anti-inflammatory effect on rat when compared with standard preparation of Diclofenac Sodium. The present work suggest that the ethanol:water extract of *Mangifera indica* Linn can be successfully used as a topical preparation. Topical gel formulation using Carbapol 934p polymer shows good stability on storage and also encouraging drug release profile and anti-inflammatory action.

Keywords: *Mangifera indica* Linn; Herbal gel; HPMC; carbopol934; Ethanol:Water

INTRODUCTION

The use of medicinal plants as herbal remedies to prevent and cure several ailments differs from community to community (Sharif MDM and Banik GR., 2006). The advent of science into the search for antibiotics largely depends on some of these medicinal plants as raw materials. For many years, medicine had depended exclusively on leaves, flowers and barks of plants; only recently have synthetic drugs come into use and in many instances, these are carbon copies of chemicals identified in plants. According to WHO, a medicinal plant is any plant which in one or more of its organs, contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Junaid SA., et al., 2006; Franklin TJ and Snow CA., 1989). At present nearly 30% or more of the modern pharmacological drugs are derived directly or indirectly form plants and in Homeopathic or Ayurvedic medicines, medicinal plants, their parts and extracts dominate the scenes. Infectious diseases continue to be the major concern for health institutions, pharmaceutical companies and governments all over the world (accounting for over 50, 000 deaths every day), especially with the current increasing trends of multidrug resistance among emerging and re-emerging bacterial pathogens to the available modern drugs or antibiotics (Prescott L., et al., 2002) . It is therefore very necessary to search for newer antibiotic sources. *Mangifera indica* L. (Family: Anacardiaceae) is commonly called *mango* (English), *manako* (Hawai'i), *manggo'am* (Fiji), *tharyetthi* (Myanmar), *mangot*, *mangue or manguier* (French), *aam*, *am* or *amb* (Hindi), *bobbie manja*, *kanjanna manja*, *magg*, *manggaboom* or *manja* (Dutch), *mamung* (Thailand), *manga* or *mango* (Spanish), *manga* (Portuguese), *manga*, *mempelam* or *ampelam* (Malaysia), *mangga* or *mempelamn* (Indonesia), *Mangobaum* (German), *paho* (Philippines) and *xoài* (Vietnam), *mongoro* (Yoruba, Nigeria), mangolo (Igbo, Nigeria) and mangoro (Hausa, Nigeria) (Pretorius CJ and Watt E., 2001; Doughari JH et al., 2007; Emeruwa AC., 1991). The genus *Mangifera* originates from the Asia, and is found greatly cultivated in Sumatra, Java, Borneo, Peninsula, Malay, India and Myanmar and practically in every tropical and sub tropical country with India having the largest area for its cultivation (Emeruwa AC., 1991).

Other common species of this genus include *Mangifera gedebe, Mangifera minor*, and *Mangifera mucronulata*. The tree is large and tall (up to 40 m) with a rounded canopy or foliage with leathery leaves and big fleshy edible drupes as fruit (Neon B., 1984). The fruits are eaten, and used for juice and wine production. Traditionally the mango plant has medicinal applications. In Côte d'Ivoire the leaf-decoction is used as a febrifuge. The bark infusion has been used as gargle to treat mouth infections in children (Tonga). In view of the importance of *M. indica* in ethnobotany as health remedy (Kubmarawa D et al., 2007).

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Received on: 08-03-2013
Revised on: 16-03-2013
Accepted on: 19-03-2013

MATERIALS AND METHODS

Materials

Carbopol 934P was obtained from Loba Chem. Pvt. Ltd, Mumbai. Methyl paraben, propyl paraben were obtained from Hi Media Laboratories. Triethanolamine was obtained from Nice Chemicals Pvt. Ltd, Mumbai. Sodium metabisulphite was obtained from SD fine chemicals, Mumbai.

Plant materials

The bark of *Mangifera Indica L*. chosen for the present investigation were collected in the months of July 2009 Nov. 2009. The stem bark parts were separated, cleaned, air dried, and grounded into powder. The powdered material was passed through sieve no. 40 and were later air-dried and stored in an air-tight container for further use.

Preparation of Herbal Extract

About 500 gm of air dried and coarsely powdered material of *Mangifera Indica* L. was extracted with (ethanol:water 80:20) for 24hrs in a Soxhalate apparatus. Then the extract was filtered with muslin cloth and evaporated under reduced pressure and dried. The fine powdered dried extract was used for further experiment.

Formulation of gel

Eight different formulations were prepared using different concentration of Carbopol 934 and HPMC separately. Accurately weighed Carbopol/HPMC was taken in a beaker and dispersed in 50ml of distilled water with constant stirring using a mechanical stirrer for 30 min at 1200 rpm. After all the Carbopol/HPMC was dispersed, propylene glycol was added with constant stirring. To the above extract preservatives were added and mixed well. 10ml of triethanolamine was added to bring the pH close to neutral which results to a consistent gel (Table-1).

EVALUATION

The above formulated herbal gel of *Magnifera indica* Linn were subjected to evaluation for the following parameters as per the method described below (Gupta GD. and Gound RS et al., 1999):

A. pH

The pH of the various gel formulations was determined by using digital pH meter (Table 2).

B. Spreadability

It was determined by wooden block and glass slide apparatus. About 20g of the gel was added to the pan and the time was noted for upper slide (movable) to separate completely from the fixed slides.

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

The results are given in Table 2.

C. Homogeneity

All batches of the gels were tested for homogeneity by visual inspection after they were kept in the containers for 24hrs. They were tested for their appearance and presence of any aggregates (Table 2).

D. Drug content

100mg each of the prepared gel and a marketed gel sample were taken separately and dissolved in 80ml of phosphate buffer of pH 6.8 in a 100ml of vol. flask. The volumetric flask containing gel solution was shaken for 2hr on mechanical shaker in order to get complete solubility of drug and made up the volume with phosphate buffer pH 6.8 to 100ml. This solution was filtered and estimated spectrophotometrically at 276nm using phosphate buffer (pH 6.8) as blank (Table 2).

E. *In vitro* release studies

The hairless albino rat skin obtained from the discards of the animal sacrifice at the pharmacology department in the college was used. The skin was soaked in 0.32 N ammonium hydroxide solution for 30 to 35 minutes to remove subcutaneous fat and hair. The skin was rinsed well with saline followed by distilled water.

Franz diffusion cell was used for permeability study: 1 g of the gel was uniformly spread over the rat skin membrane and tied over the donor compartment. The skin was placed with stratum corneum facing the donor compartment and the dermis facing the receptor compartment contain100ml distilled water. At hourly intervals, 5ml of sample was withdrawn from the receptor and replaced with fresh 5ml distilled water. The 5 ml withdrawn sample was made up to 25 ml with distilled and the absorbance was recorded at 280nm. The receptor medium was magnetically stirred for uniform distribution and was maintained at a temperature of 37°C ± 0.2°C (Sera UV. and Ramana MV et al., 2006). The results were given in Table 3, Fig 1.

F. Stability studies

The batch no F-4 was showing the results meeting the standard specification requirement for gel and hence considered to be the optimised batch. Batch No. F-4 was subjected to stability testing for three months as per ICH norms at a temperature of30°±2°C/ 65% ± 5% RH and 40° ±2°C/ 75% ± 5% RH for intermediate and accelerated stability. The formulations were analyzed

for the change in colour, appearance spredability, pH and assay (ICH Guidelines et al., 2003)

G. Rheological Studies

Rheological properties of a pharmaceutical system can influence the selection of processing equipment used in the materials according to type of flow and determination. It is customary to place them in one of the two categories: Newtonian or Non-Newtonian systems. The choice depends on whether or not their flow properties are in accord with Newton's law of flow (Sinko PJ).

Viscosity determinations of the prepared formulations were carried out on a Brooke field viscometer. The viscosity of the sample were measured at different angular velocities at a temp of 37±1°C. The average of the three readings were used to calculate the viscosity.

H. *In vivo* Anti-inflammatory activity

Healthy adult albino rats of Wistar strain weighing 180-250g were used for this study. Animals were housed at temperature of 24±2°C and relative humidity of 30-70%. A 12:12 light: day cycle was followed. All the animals were allowed to free access to water and fed with standard commercial pelleted rat chaw. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (IAEC/VIPER/Ph.D/2011-12-01). This method was based on plethysmographic measurement of carrageenan-induced acute rat paw odema produced by sub plantar injection of carrageenan in hind paw of the rat. This method described by Wilhmi and Domenjoz et al. was used for measuring the paw volume. This procedure of measuring the paw volume required two operators, one for dipping the paw of the rat in potassium permanganate solution and another to measure and record the paw volume simultaneously.

Anti-inflammatory activity was measured according to the method of winter et al. Rats were distributed into 3 groups of 6 animals each. One group served as control and the other two as treatment groups. Treatment groups were given prior treatment (50mg each of diclofenac sodium gel and *Mangifera indica* Linn. extract gel respectively) to the top of the right hand paws of rats with gentle rubbing for 15 sec. After 1 hr, 0.1ml of a 1% w/v suspension of carrageenan in normal saline was injected in to the subplanter region of right hind paw of all the control and treated rats (Garrido G et al., 2001; Griswold DE et al., 1998).

RESULTS AND DISCUSSION

Herbal gel prepared using 2% *Magnifera indica* Linn. extract alcohol-water(80-20) with two different polymers namely carbopol 934P and HPMC separately. Eight batches were prepared and found that all the formulations were brown transparent in colour with uniform consistency.

Gels prepared using Carbople934P had a pH value between 6,4 to 7.0 whereas HPMC based gels revealed a pH value between 6.8 to 7.2. The gels which have pH value in the range of 5.5 to 7.5 are most ideal, as they near the pH of the skin and cause practically no

Table 1: Composition of *Magnifera indica* Linn extract gel formulations

Batch No	Dry Extract % w/w	Carbopol 934P	HPMC (gm)	Propylene Glycol (gm)	Sod. Metabisulphite	Triethanolamine	Distilled water Up to (g)
F1	2	0.5	-	5	0.1	10	100
F2	2	1	-	5	0.1	10	100
F3	2	1.5	-	5	0.1	10	100
F4	2	2.5	-	5	0.1	10	100
F5	2	-	0.5	5	0.1	10	100
F6	2	-	1	5	0.1	10	100
F7	2	-	1.5	5	0.1	10	100
F8	2	-	2.5	5	0.1	10	100

Table 2: Evaluation results of *Magnifera indica* Linn bark extract topical gel

Sr. No.	Batch No.	pH	Viscosity (cps)	Spreadability (gm .cm/sec)	Drug Content	Skin Irritation
1	F1	7.0	32170	37.50	92.26	Nil
2	F2	6.8	48240	25.46	90.23	Nil
3	F3	6.4	53180	22.12	89.47	Nil
4	F4	7.0	64250	48.47	95.6	Nil
5	F5	6.8	55380	30.0	91.41	Nil
6	F6	7.0	65640	24.07	91.04	Nil
7	F7	7.2	78720	17.79	89.23	Nil
8	F8	7.0	81548	16.44	92.48	Nil

Nature of all formulated gel is brown transparent semisolid

Table 3: Drug release study of *Mangifera indica* Linn. bark extract topical gel

Time (hours)	% Drug release							
	F1	F2	F3	F4	F5	F6	F7	F8
1	36	32	41	43	33	37	33	40
2	55	52	57.55	59	52	56	51.69	56
3	61	58.5	63	65.45	58.12	62.22	57.12	62.47
4	68	64.8	67.43	69.32	65.34	69.31	63.75	66.75
5	74.5	68.89	71.33	73	71.54	75.61	67.21	70.00
6	77.43	71.9	76.66	78.89	75.65	7829	70.19	75.32
7	80.14	73.3	81.45	83.55	77.36	81.02	72	82.17
8	82.5	75.5	86.90	89.95	80	83.22	74.99	84.31
9	84	77.65	89.33	92	82.87	85.09	78	87.29
10	92.26	90.23	89.47	95.6	91.41	91.04	89.23	92.48

Table 4: Stability data of *Mangifera indica* Linn. bark extract topical gel of batch No. F4

Intermediate stability					
30°C ± 2°C/ 65% ± 5% RH	**Colour**	**Appearance**	**Spreadability (gm.cm/sec)**	**pH**	**Assay**
Initial	Brownish	Transparent	48.47	7.0	95.6
1 month	NC	NC	47.91	6.97	93.0
2 month	NC	NC	46.41	6.94	93.2
3 month	NC	NC	46.38	6.93	93.0
Accelerated stability					
40^0 C ± 2^0C/ 75% ± 5% RH	**Colour**	**Appearance**	**Spreadability (gm.cm/sec)**	**pH**	**Assay**
Initial	Brownish	Transparent	48.47	7.0	95.6
1 month	NC	NC	47.86	6.90	94.0
2 month	NC	NC	46.79	6.87	94.3
3 month	NC	NC	46.58	6.93	94.0

Table 5: Effect of *Mangifera indica* L. Extract topical gel on carragenan induced paw oedema indicating oedema volume & oedema inhibition (Batch No. F4)

Group	Dose	1 hr	3 hr	5 hr
		EV EI	EV EI	EV EI
Control	---	2.40±0.03 ---	2.65±0.02 ---	2.58±0.03 ---
Diclofenac gel	50mg/kg	1.52±0.03 36.66	1.05±0.02** 60.03	1.20±0.02* 53.48
Gel	50mg/kg	1.42±0.07 40.33	0.99±0.02*** 62.64	1.09±0.02* 57.75

Values are expressed as mean ± SEM (n=6).

EV – Oedema volume EI – Oedema inhibition.

* Significant at p< 0.05, ** highly significant at p<0.01, *** Very highly significant at p<0.001

irritation. *In-vitro* release study for the drug across hairless albino rat skin with Carbopol934P based gels revealed a % CDR of 92.26, 90.23, 89.47 and 95.6, while HPMC based gels were found a % CDR of 91.41, 91.04, 89.23 and 92.48 at the end of 10 hrs (fig.3). The release of drug from both the 8 formulations followed zero-order kinetics. The viscosity (cps) of the prepared gel with Carbopol934P was 32170 to 64250cps while HPMC based gels was found to be 55380 to 81548cps. A creep curve (Fig 2) obtained by analysing the gel shows the nature of viscoelastic material showing both viscosity in the liquid state and elasticity in the solid state. The rheogram (Fig. 1) of the prepared gel exhibits pseudoplasticity (Non Newtonian nature). The pseudoplastic nature renders its suitable for formulation using various rotational equipment. The creep curve of the gel indicates that it has proper viscoelastic nature which is prime requirement for a good gel. The Spreadability (gm.cm/sec) of the Carbopol934P based gels were 22.12 to 48.47gm.cm/sec while HPMC based gels viscosity was 16.44 to 30.30 gm.cm/sec. However it is observed that there is marked increase in viscosity range with HPMC verses Carbopol 934 P.

Taking all the parameters into consideration the F4 Batch is considered as optimised one and is chosen for stability study as per ICH guidelines. Stability studies for gels revealed good physical stability, colour and consistency for the optimised formulations along with drug content.

For anti-inflammatory activity oedema volume was measured in all the three pre-treated groups with gel at 1hr, 3hr and 5hr interval after carrageenan challenge and also calculated percentage of inhibition in all the groups. *Mangifera indica* L. extract gel exhibited significant reduction of oedema. The percent reduction of

oedema with *Mangifera indica* L. gel was found to be 62.64% which is marginally higher than the marketed Diclofenac gel having 60.03%. The results were given in table no 5.

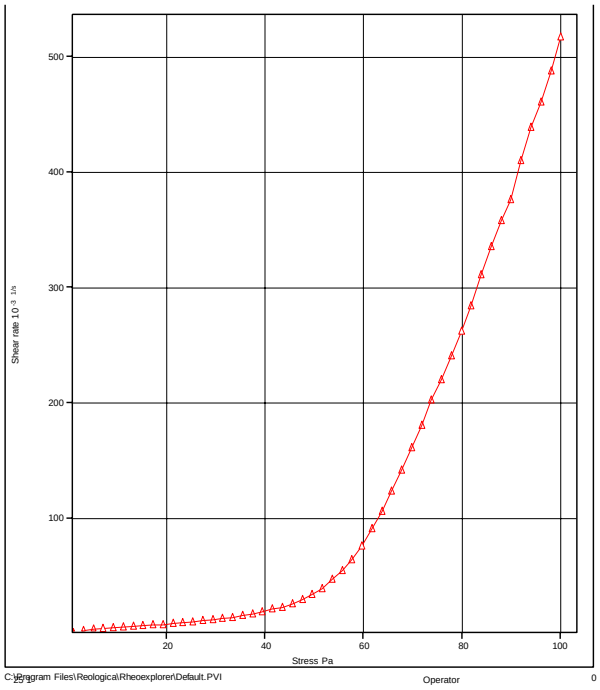

Figure 1: Rheogram of *Mangifera indica* L. Bark extract topical Gel showing pseudoplastic flow. (Batch No. F4)

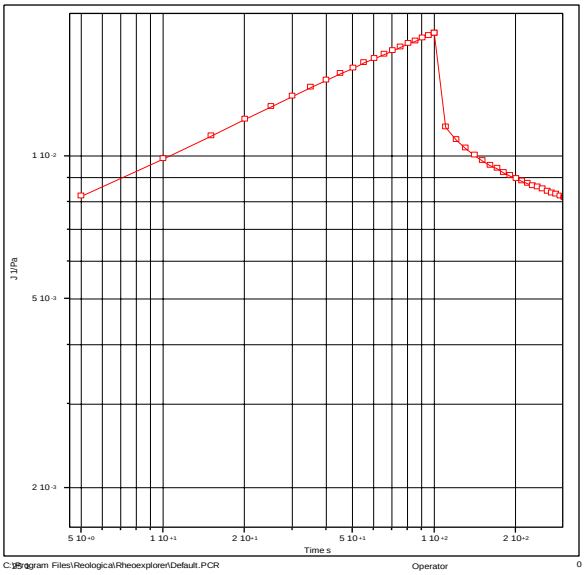

Figure 2: Creep Curve: Showing viscoelastic nature of Gel. (Batch No. F4)

CONCLUSION

In Indian system of medicine majority of herbal products are made by using crude plant or portion of plant parts and their extracts. The dried bark extract of matured *Mangifera indica* L. plant belongs to family *Anacardiaceae* was taken for this present study and formulated for the topical gel and its Properties. The gel prepared using *Mangifera indica* L. bark hydroalchoholic extract was found to have good gel characteristics with respect to homogeneity, spredability, stability and anti-inflammatory effect was comparable. Hence from the above study it can be concluded that the hydroalcholholic dried extract of *Mangifera indica* L. plant in pharmaceutical dosage form gives comparable anti-inflammatory activity and can be of commercial significance.

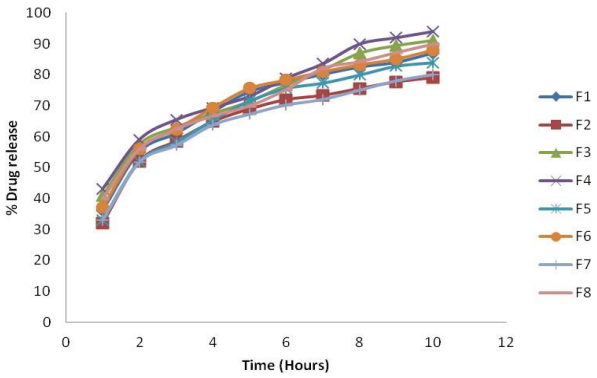

Figure 3: Percentage drug release profile of *Magnifera indica* Linn extract herbal gel batches

ACKNOWLEDGEMENT

The authors are grateful to Dr. S. Parasuraman, M.Pharm, PhD, JIPMER, Pondicherry-06 and Dr. E.P. Kumar, Head, Dept. of Pharmaceutical Sciences, Karpagam University, Coimbatore-641021, Tamilnadu, India for their guidance and support.

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