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Article

Formulation development of anti-rheumatoid gel of *Saraca asoca* (Roxb.) De Wilde hydroalcoholic extract containing eucalyptus oil and peppermint oil as penetration enhancer

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In the present research investigation, various concentrations of hydro-alcoholic extract of *Saraca asoca* (Roxb.) De Wilde (family: Caesalpinaceae) dried bark and carbopol polymer at different temperature ranges were optimized for the preparation of gel formulation. Natural penetration enhancers, *v.i.z.*, eucalyptus oil and peppermint oil were incorporated separately in the extract based gel formulations to study the rate of drug permeation across egg membrane, using franz diffusion cell. *In vitro* anti-arthritis potential of the formulations was also studied using inhibition of albumin denaturation, antiproteinase activity and membrane stabilization method. As per the results of current study, it is established that *S. asoca* dried bark hydroalcoholic extract based gel prepared using peppermint oil as penetration enhancer exhibited good permeation rate of 8.48% at the end of 3 h. The percentage inhibition of proteins by antiproteinase method at concentration of 50 µg/ml was 50.01±1.00% which was close to 53.92±0.99% as shown by the standard drug, Diclofenac. Also, the percent protein inhibition determined using membrane stabilization method was found to be 49.70±1.00%, however, it was 63.32±0.94% for the standard drug, Diclofenac. Hence, it is concluded that peppermint oil may act as a good candidate for the preparation of potent anti-rheumatic gel preparations.

Keywords: Extract. *In vitro.* Penetration enhancer. Rheumatoid arthritis, *Saraca asoca* (Roxb.) De Wilde.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic inflammatory and chronic disease that mainly affects periarticular tissues, joints and produces various functional disabilities like cartilage destruction and crippling deformities. RA patients are suffering from declined functional ability due to inability in preventing cartilage breakdown and joint destruction. Pro-inflammatory molecules released by macrophages such as reactive oxygen species and eicosanoids such as prostaglandin, cytokines and leukotrienes are responsible for this inflammatory disease. For chronic inflammatory conditions, cyclooxygenase (COX) and liquid oxygen (LOX) are potential targets and by inhibiting these enzymes, modulation of arachidonic acid metabolisms are regulated (Reddy, Rao, Lakshmi, 2014). RA patients are suffering from declined functional ability due to inability in preventing cartilage breakdown and joint destruction. Other major reason responsible behind RA is the secretion of lysosomal enzymes during inflammation leading to tissue injury due to damage of macromolecules and lipid peroxidation of cellular membranes.

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Saraca asoca (Roxb.) De Wilde, commonly known as 'Ashoka tree', belonging to family Caesalpinaceae, is mainly found in Myanmar Malayan, Srilanka, Peninsula and Bangladesh. The paste of this bark is used in the management of uterine inertia, uterine pain, urinary calculus and dysurea. In case of leucorrhoea, boiled (in milk and water) ashoka bark is used for treatment. The boiled ashoka bark is also used in the management of various diseases like gynecological disorders, menorrhagia etc. (Pradhan *et al*., 2009). It contains polyphenolic compounds which can be useful in treatment of arthritis, in essence inhibition of necrosis factor (TNF-α), interleukin-1β (IL-1β) and suppression of activation of necrosis factor-κB, NF-κB (Abidin *et al*., 2016).

For the treatment of arthritis, transdermal route is preferred over conventional route of drug administration as it offers an alternative pathway for systemic drug delivery. Niosomal gel of ursolic acid has been reported to be a promising alternative to conventional therapy for safe and efficient treatment of arthritis and musculoskeletal disorders (Jamal *et al*., 2015). However, penetration enhancers from natural origin have become popular due to lower cost depending on the type of extraction process used and sustainable mass production from renewable resources. In this study, natural penetration enhancers has been added to optimized gel containing *S. asoca* bark extract to enhance the potential of transdermal drug delivery system.

MATERIAL AND METHODS

Diclofenac was received as gift sample from Panacea Biotech Ltd. (India). Carbopol and triethanolamine were supplied by Merck Ltd. (India). Eucalyptus oil and peppermint oil were procured from Yucca Enterprises, Mumbai (India). All other chemicals were of analytical reagent grade and used as received.

Plant material

The dried bark of *Saraca asoca* (Roxb.) De Wilde was selected and used for the present research study.

METHODS

Collection and authentication of selected plant

Saraca asoca (Roxb.) De Wilde bark was collected from herbal garden of Maharshi Dayanand University, Rohtak and authenticated by Professor B. D. Vashistha, Department of Botany, Kurukshetra University, Kurukshetra (Voucher specimen no. KUK/MDU/IPS-41).

Preparation of extract

The collected *S. asoca* bark was dried in oven below 40°C. The dried bark was ground into coarse powder using a mechanical grinder (Bhilana *et al*., 2018) and stored in fresh plastic polythene bags. 100 g of powdered bark was extracted with hydroalcohol (water:alcohol:: 50:50) using soxhlet apparatus for 7 h. The resulting extract was cooled and filtered. The filtrate was concentrated using rotary vacuum evaporator, dried using lyophilizer and stored in desiccator for further use (Mukhopadhyay, Nath, 2011).

Optimization and development of *S. asoca* gel (SAG)

Experimental Design

For formulation optimization, recent version of Design Expert Software was used. Variables and invariables used in the present study are given in Table I.

Using 2^3 full factorial design, a batch of eight formulations was designed and analyzed further.

TABLE I - 2^3 full factorial design matrix showing composition of different batches of SAG

Gel Code	Factor 1 (*S. asoca* extract in g)	Factor 2 (Carbopol Concentration in g)	Factor3 (Temperature in °C)	Sodium benzoate (g)	Triethanolamine (ml)	Distilled water (ml)
SAG 1	2 (Low)	2.5 (High)	70 (High)	1	0.5	q. s
SAG 2	2 (Low)	0.5 (Low)	40 (Low)	1	0.5	q. s
SAG 3	8 (High)	0.5 (Low)	40 (Low)	1	0.5	q. s
SAG 4	8 (High)	0.5 (Low)	70 (High)	1	0.5	q. s
SAG 5	8 (High)	2.5 (High)	40 (Low)	1	0.5	q. s
SAG 6	2 (Low)	2.5 (High)	40 (Low)	1	0.5	q. s
SAG 7	8 (High)	2.5 (High)	70 (High)	1	0.5	q. s
SAG 8	2 (Low)	0.5 (Low)	70 (High)	1	0.5	q. s

Here, q.s. implies quantity sufficient

Method of formulation of *S. asoca* gel

The dried hydroalcoholic extract of *S. asoca* bark was used for preparing the gel.

The different gel base formulations were prepared by dissolving carbopol 940 in 20 ml distilled water with continuous stirring by magnetic stirrer at different temperature range as given in Table I. The hydro alcoholic extract of *S. asoca* was dissolved in 10 ml distilled water separately. Then, the solution of extract was mixed in the solution of carbopol with continuous stirring. After that, 1 gm of sodium benzoate was added as preservative and volume was made up to 50 ml with distilled water. pH was adjusted by adding few drops of triethanolamine and the prepared gel was stored in a cool and dry place (Aiyalu, Govindaran, Ramasamy, 2016). All the eight batches of formulations were evaluated for their *in vitro* membrane permeation study using a franz -diffusion cell in order to optimize and get the best batch on the basis of permeability results.

In-vitro membrane permeability studies

Eight batches of *S. asoca* gel formulations were subjected to drug release studies using egg membrane with a permeation area of 5.12 cm^2. 55 ml of phosphate buffer (pH 7.4) was taken in receptor compartment, and then 2 g of gel was applied on membrane (Singh *et al*., 2015). The donor phase was kept in contact with receptor phase and the temperature was maintained at 37±0.1°C (Goyal *et al*., 2011). The entire assembly was placed on a magnetic stirrer. 2.5 ml of sample was withdrawn at a time interval of 30, 60, 90, 120, 150 and 180 min and replaced with fresh phosphate buffer, pH 7.4 (Dhiman, Arun, Ahmad, 2016). The withdrawn sample was analyzed spectrophotometrically at 280 nm.

The results were optimized and the best suitable formulation on the basis of maximum membrane permeation was selected. The concentration of extract, carbopol and temperature was thereafter considered optimized and used for further study.

Formulation of *S. asoca* gel, SAG with natural penetration enhancers (using Eucalyptus oil, SAGE and Peppermint oil, SAGP)

Essential oils or volatile oils are found in flower, fruits, leaves and root of various plants and are known to have skin penetration enhancing properties. It has been reported that volatile oils may increase the

penetration rate at a lower concentration (Fox *et al*., 2011). 2^3 full factorial design was used for optimization and development of gel with penetration enhancers (eucalyptus and peppermint oil) taking three variable *v.i.z.* concentration of enhancer, heating time and stirring speed as mentioned in Table II. The gel base formulation was prepared by using previously optimized values of *S. asoca* extract, carbopol and temperature. 1.5 gm of carbopol 940 was dissolved in 20 ml distilled water with continuous stirring on a magnetic stirrer at temperature 55°C. Meanwhile, 5 gm of hydroalcoholic extract of *S. asoca* was dissolved in 10 ml distilled water separately. Then the solution of extract was mixed in carbopol solution with continuous stirring. 1g of sodium benzoate was added as a preservative and volume was made up with water upto 50 ml. Then penetration enhancer was added in the formulation followed by pH adjustment by adding few drops (0.5 ml) of triethanolamine. The prepared gel was stored at cool and dry place at room temperature (Aiyalu, Govindaran, Ramasamy, 2016).

Total of sixteen formulations were made using penetration enhancers, eight batches containing SAGE i.e. *S. asoca* gel with eucalyptus oil and eight batches of SAGP i.e. *S. asoca* gel with peppermint oil. These formulations were optimized on the basis of best permeability across egg membrane.

TABLE II - Composition of various batches of SAG with penetration enhancer

Gel Code	Conc. of extract (g)	Conc. of carbopol (g)	Temperature (°C)	Conc. of Penetration enhancer (Eucalyptus oil or peppermint oil) in ml	Heating time (Min.)	Stirring speed (rpm)	Sodium benzoate (g)	Triethanolamine (ml)	Distilled water (ml)
SAGE 1 / SAGP 1	5	1.5	55	2	40	1250	1	0.5	q. s
SAGE 2 / SAGP 2	5	1.5	55	2	25	250	1	0.5	q. s
SAGE 3 / SAGP3	5	1.5	55	10	25	250	1	0.5	q. s
SAGE 4 / SAGP 4	5	1.5	55	10	25	1250	1	0.5	q. s
SAGE 5 / SAGP 5	5	1.5	55	10	40	250	1	0.5	q. s
SAGE 6 / SAGP 6	5	1.5	55	2	40	250	1	0.5	q. s
SAGE 7 / SAGP 7	5	1.5	55	10	40	1250	1	0.5	q. s
SAGE 8/ / SAGP 8	5	1.5	55	2	25	1250	1	0.5	q. s

Here, conc. implies concentration; q.s implies quantity sufficient

SAGE refers to *S. asoca* gel with eucalyptus oil; SAGP refers to *S. asoca* gel with peppermint oil

Evaluation of *S. asoca* gel (SAG), *S. asoca* gel with eucalyptus oil (SAGE) and *S. asoca* gel with peppermint oil (SAGP) for *in vitro* anti- rheumatoid arthritis activity

In vitro models, *v.i.z.* inhibition of albumin denaturation, antiproteinase action and membrane stabilization were used to evaluate the optimized gel formulations prepared with and without penetration enhancers. The results were compared with standard drug, diclofenac.

Inhibition of albumin denaturation

The anti- rheumatoid athritis activity of gel formulation of SAG, SAGE and SAGP was determined by inhibition of protein denaturation technique given by Hossain *et al*., (2015).

Antiproteinase Action

The anti- rheumatoid arthritis activity of gel formulations of SAG, SAGE and SAGP was evaluated on the basis of their antiproteinase action according to the method given by Leelaprakash and Mohan (2011).

Membrane stabilization

The anti- rheumatoid arthritis activity of all the three optimized formulations (SAG, SAGE and SAGP) were determined on the basis of membrane stabilization method given by Leelaprakash and Mohan (2011).

RESULTS AND DISCUSSION

The anti rheumatoid arthritis gel was optimized and developed using 2^3 full factorial design. Three variables were taken and various formulations were prepared on the basis of these variables and optimized by their response of percent cumulative drug release. The three batches of eight formulations each were prepared *v.i.z.* without enhancers and with two selected natural enhancers i.e. using peppermint oil and eucalyptus oil. All these formulations were optimized on the basis of cumulative drug release and the optimized gel formulations were further evaluated for their physicochemical properties and *in vitro* anti rheumatoid arthritis activity.

The yield of hydroalcoholic extract of *Saraca asoca* (Roxb.) De Wilde dried bark was found to be 15.4 % w/w. Results of percentage permeation rate of SAG, SAGE and SAGP are given in Table III.

The results revealed that SAG 7, SAGE 5 and SAGP 3 were the best suitable formulations with maximum rate of drug penetration. The optimized batches of SAG, SAGE and SAGP were further evaluated for their cumulative drug release behavior, the results of which are given in Table IV.

TABLE III - Permeability study results of SAG, SAGE and SAGP

Rate of Permeation (%)	Codes of *S. asoca* dried bark hydroalcoholic extract gel (SAG)							
	SAG 1	SAG 2	SAG 3	SAG 4	SAG 5	SAG 6	SAG 7	SAG 8
	7.72	6.36	1.36	7.72	6.36	3.1	8.18	5.0
	Codes of *S. asoca* dried bark hydroalcoholic extract gel with eucalyptus oil (SAGE)							
	SAGE 1	SAGE 2	SAGE 3	SAGE 4	SAGE 5	SAGE 6	SAGE 7	SAGE 8
	1.39	8.78	9.04	6.50	7.68	7.24	1.55	7.80
	Codes of *S. asoca* dried bark hydroalcoholic extract gel with peppermint oil (SAGP)							
	SAGP 1	SAGP 2	SAGP 3	SAGP 4	SAGP 5	SAGP 6	SAGP 7	SAGP 8
	3.84	5.29	5.60	3.57	8.48	2.12	1.68	7.27

TABLE IV - Cumulative drug release of optimized formulations, SAG, SAGE and SAGP

S. No.	Time (min)	Percent Cumulative drug release (%CDR)		
		Optimized gel formulations		
		SAG 7	SAGE 3	SAGP 5
1	30	2.27	4.54	2.54
2	60	4.54	8.76	2.67
3	90	5.00	8.77	2.86
4	120	6.36	9.02	6.50
5	150	7.72	8.80	7.76
6	180	8.18	9.04	8.48

Evaluation of *in - vitro* anti rheumatoid arthritis activity

The results of percentage inhibition of proteins calculated for optimized SAG, SAGE and SAGP considering three *in vitro* methods, *v.i.z.*, inhibition of albumin denaturation method, antiproteinase activity and membrane stabilization technique are mentioned in Table V. Results were compared with standard drug, Diclofenac.

From *in vitro* study results, it is evident that the optimized formulation of *S. asoca* dried bark hydroalcoholic extract gel with added peppermint oil (SAGP5) showed enhanced percentage protein inhibition, when compared with other two optimized formulations (SAG 7 and SAGE 3). Denaturation of protein is alteration in electrostatic hydrogen, hydrophobic and disulphide bonding and one of the well renowned causes of inflammation and production of auto-antigens in certain arthritic disease. The increments in absorbance of plant extract indicated the stabilization of albumin protein. This anti-denaturation effect was further supported by change in viscosities as viscosities of protein solutions was also increased on protein denaturation. The plant extract might have inhibited the processes, which may stimulate or enrich the efflux of these intracellular components.

TABLE V - Results of *in vitro* anti arthritic activity of optimized formulations, SAG, SAGE and SAGP

Concentration (µg/ml)	Percentage inhibition of proteins											
	Protein denaturation method				Antiproteinase method				Membrane stabilization method			
	SAG 7	SAGE 3	SAGP 5	Diclofenac	SAG 7	SAGE 3	SAGP 5	Diclofenac	SAG 7	SAGE 3	SAGP 5	Diclofenac
10	10.30±0.97	53.33±0.99	65.00±0.93	68.33±1.00	10.42±0.98	19.79±0.96	34.12±1.03	42.83±0.60	13.02±0.79	24.72±1.03	29.79±0.59	52.23±0.70
20	13.33±1.00	58.33±0.98	70.33±0.97	73.33±0.99	12.11±0.75	23.20±0.86	39.41±0.99	44.88±0.79	16.61±0.85	29.81±0.79	33.60±0.85	54.68±0.70
30	16.66±0.97	65.00±0.93	76.66±0.99	78.33±0.98	14.33±0.97	26.45±0.96	44.70±0.72	46.07±0.88	19.53±0.97	34.90±0.72	39.65±0.89	57.57±0.78
40	21.66±0.98	71.66±0.99	81.66±098	83.33±0.96	18.08±0.71	30.03±0.77	47.01±0.65	49.65±0.96	23.68±0.81	39.61±0.75	43.53±0.77	60.05±0.98
50	28.33±0.99	81.66±0.99	85.30±0.97	88.66±0.99	20.64±0.93	34.30±1.00	50.01±1.00	53.92±0.99	28.74±0.69	45.71±1.00	49.70±1.00	63.32±0.94

CONCLUSION

From the present research study, it is concluded that the hydro alcoholic extract based gel formulation of *S. asoca* bark has shown significant anti-arthritic activity as supported by *in vitro* models. Peppermint oil incorporated gel formulation of *S. asoca* dried bark extract showed better permeation rate and anti-arthritic activity as compared to the eucalyptus oil incorporated gel formulation of *S. asoca* dried bark extract and gel formulation of *S. asoca* dried bark extract without added penetration enhancers. However, the significant anti-rheumatoid activity may be attributed to the phenolic compounds active constituents present in the hydroalcoholic extract of *S. asoca* (Roxb.) De Wilde which was further enhanced upon addition of peppermint oil and eucalyptus oil. Peppermint oil acted as better penetration enhancer than eucalyptus oil when incorporated in transdermal drug delivery system containing *S. asooca* dried bark hydroalcoholic extract.

CONFLICTS OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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