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Design and development of the herbal formulation for the treatment of over-active bladder

Kunal K Mehta, Vishal Gupta N, Gowda DV*, Manohar M

Department of Pharmaceutics, JSS College of Pharmacy, Sri Shivarathreeshwara Nagara, Mysuru, JSS Academy of Higher Education and Research, JSS Medical Institutions Campus, Sri Shivarathreeshwara Nagara, Mysuru – 570015, Karnataka, India

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ABSTRACT

The Objective of the study was to formulate a novel, topical hydrogel system containing pumpkin seed extract for the Over Active Bladder treatment. Formulations were prepared using pumpkin seed extract (1.0% w/v) and varying concentration of Carbopol (0.5%-1.25% w/v) was used as a gel base. The prepared topical gels were evaluated for Drug content, pH, Physical appearance, viscosity, content uniformity, *and In vitro* drug diffusion. The results demonstrated that different formulations of Pumpkin seed extract (1%) in consolidation with carbopol 940 exhibited in the gel preparation that was considerably uniform and stable. The spreadability of an optimized formulation was found to be 1.5cm and the% drug diffusion at the 6thhour was found to be 56.35%. The drug content was found to be 94.5%. Viscosity was found to be 3.78 Lakhs cps. The pHwas found to be 6.80. Drug content & Physical Appearance revealed that the formulations were stable. Moreover, it was concluded that hydrogel containing herbal extract is safe and effective for treatment of over Active Bladder (OAB).

* Corresponding Author

Name: Dr. D.V.Gowda
Phone: +91-9663162455
Email: dvgowda@jssuni.edu.in

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INTRODUCTION

Overactive bladder a degenerative and disabling syndrome, i.e. defined by urinary necessity with or without involuntary excretion, commonly in combination with urinary regularity and nocturia (Abrams P *et al.,* 2002). It is equally seen in women (16.9 %)and men (16 %); however, women suffer from OAB. The women experiencing urge urinary urge incontinence monthly or more often is 5-10%. The prevalence of OAB is almost 20 % in those over

Some conditions are associated with the symptoms and signs of OAB including functional and behavioural conditions, neurological conditions, lower urinary tract conditions, and use of various other medications. Also, OAB and urinary incontinence 60 years of age (Tang, D.H *et al.,* 2014)

are associated with skin infections and irritations (Steward *et al.,* 2003).

In the comfort zone, the force within the bladder is less than that of the urethral aid. In a normal being, the bladder can hold a capacity of about 350 and 500 ml with the first urge to urinate occurs when the bladder contains an amount of about 200 ml. Maturation occurs during a series of events that starts with a reduction in urethral resistance. Subsequently, the bladder is surrounded by the smooth muscle layer contracts and results in emptying of the bladder. This series of events starts when the bladder's stretch receptors are activated thereby sending signals from the spinal cord to brain centres, resulting in the sensation of urge due to increased bladder filling (Keam *et al.,* 2004). The signs of OAB are usually related by overactivity of the detrusor muscle as it contracts spastically, oc-

casionally without a recognized source. This results in sustained high bladder pressure and or urge incontinence depending on the sphincter reaction (Ouslander *et al.,* 2004; Brady *et al.,* 2001).

The mechanism by which these neurotransmitters regulate are not only of research importance but to also provide a foundation for therapeutic intervention in patients by avoiding dysfunction. In recent times, more than a few noradrenergic, non-cholinergic intermediates which play a vital role in the physiology of lower urinary tract have been identified. Nitric oxide (NO) over the past ten years has progressively gained the importance as a major cell mediator in the lower urinary tract with a wide range of function. It is recognized as an important modulator of the cellular immune response as well as NANC neurotransmitter (Dutcher *et al.,* 2003; Berghmans *et al.,* 2000; Payne *et al.,*2000).

To treat OAB several effective formulations of antimuscarinic agents are available in the market. Although proven efficacy in the OAB, however, such agents have several limitations or adverse actions such as blurred vision, sleeping problems, dryness of mouth, constipation, diarrhoea and running nose etc. Hence to overcome all these limitations an attempt was made to formulate the topical hydrogel by using Pumpkin Seed extract for the effective treatment of Overactive Bladder. Carbopol is made of carbomers. The main work of this study is to formulate, characterize and evaluate the hydrogel containing Herbal extract for the effective treatment of OAB.

Table 1: Formulation of the gel using different concentration gelling agents

S.No	Ingredients (%w/w)	C1	F1	F2	F3	F4
1	Pumpkin seed extract	---	1	1	1	1
2	Carbopol 940	0.75	0.5	0.75	1.0	1.25
3	Glycerin	5	5	5	5	5
4	Propylene Glycol	10	10	10	10	10
5	Urea	5	5	5	5	5
6	Paraben Methyl	0.03	0.03	0.03	0.03	0.03
7	Paraben Propyl	0.01	0.01	0.01	0.01	0.01
8	TEA	QS	QS	QS	QS	QS
9	Water Up to	100	100	100	100	100

MATERIALS AND METHODS

Materials

Pumpkin Seed Extract was procured from The Local Market. Carbopol 940, Sodium Hydroxide, and Potassium Dihydrogen Phosphate procured from Loba Chemie private ltd. Propylene Glycol procured from Sigma Aldrich private ltd. Glycerin, Urea, Triethanolamine, Methyl Paraben and Propyl Paraben procured from Merck private ltd.

Formulation of the topical hydrogel of API

The different formulation was done with different types of polymers (HEC, HPC and Carbopol 940) using different concentration as shown in Table 1. The following are a combination with Carbopol 940 resulted in the best gel preparation, which was uniform and stable. The required quantity of aqueous phase (water) was weighed (Electro labs Digital balance) in a beaker and was placed on a heating mantle (Remi heating mantle) at a temperature 80°C along with stirring with an overhead stirrer (Remi magnetic stirrer 1MLH). The required amount of preservatives were weighed and added to the warm water. The aqueous phase has to be heated to make the preservatives solubilized. After the parabens are solubilized, the aqueous phase was maintained to room temperature. The water was weighed, if the water is lost in the form of vapours, the weight correction is done by adding water that was lost during heating. This was done to ensure that the proportions of phases do not change. The required quantity of propylene glycol, glycerin and urea were weighed and added to water and continued stirring at 1000rpm. The further required quantity of API was mixed to the above mixture. The optimized quantity of gelling agent (Carbopol 940) was weighed and added to water and continued stirring. Finally, the volume was made up to 100 ml by adding remaining distilled water. Triethanolamine was added dropwise to the preparation for the modification of required pH (6.8-7) to attain the required consistency of the gel. Finally, the mixture was mixed properly with stirring to form a semisolid gel after 2-3 min. The polymer absorbs water and swells to form a gel. The technique was monitored for preparation of control and sample.

Characterisation

Compatibility studies

Drug and excipients in 1:1 ratio were mixed and stored in glass vials at room temperature, 50°C and 60°C. The samples were analyzed for compatibility after 4 weeks for any physical changes like discolouration (Gupta *et al.,* 2010).

Physical appearance and homogeneity

The prepared formulations (F1-F4) were tested for homogeneity by observing visually. They were observed for uniformity of gel, aggregates, foreign matter and phase separation etc. (Gupta *et al.,* 2010).

Evaluation

Measurement of pH

The measurement pH of the topical gel is important for two reasons. One is the pH of human skin ranges from 5-7 and if the pH of the formulations is too acidic or too basic. The digital pH meter was used to measure the pH of the gel. Before measuring the pH of formulation the pH meter was calibrated by using standard buffered solutions.1gm of the gel was weighed in a beaker; to it, 100ml of DM water was added and stirred with a magnetic stirrer until it forms a uniform solution. Then the pH was recorded. (Islam *et al.,* 2004).

Measurement of viscosity

The viscosity of the gel is measured at 25°C with the help of Brookfield viscometer (LV-DVII) with spindle no.S-96 at 1 rpm and viscosity was measured in cps. The measurement of each gel was done in triplicate and the standard deviation was calculated. (Islam *et al.,* 2004).

Drug content

Accurately weighed 1gm of the gel was taken in a 100 ml volumetric flask, DM water was added to make up the volume. The flask was shaken well to break the lumps and the mixture was sonicated for 50 min with occasional shaking. The sample was maintained at room temperature and filtered through 0.45u filter. Drug concentration was determined by measuring the absorbance of the solution at 260 nm using UV-Vis spectrophotometer. (Khullar.R *et al.,* 2011). The assay was calculated by the formula:

$$\%\text{Assay}=\frac{Sample\ absorbance}{\text{S/012032 0456340178}}\times\frac{\text{S/012032 761781/30/961}}{50\ \text{=;<8 761781/30/961}}\times \text{Purity}$$

Content uniformity

Content uniformity test gives the assurance that the API in the formulation is distributed uniformly. 10 clean and dry 100ml volumetric flasks were taken. To each flask, approximately 1g of the gel was weighed carefully so that the gel does not stick to the walls of the volumetric flask. Approximately 60ml of DM water was added and the flask was shaken well to break the lumps of gel. The mixture was sonicated for 50 min with occasional shaking. The samples were cooled to room temperature, was made up to the volume with DM water and mixed well to ensure the uniformity. The sample was filtered through 0.45u filter and samples were analyzed spectrophotometrically at 260 nm. (Khullar.R *et al.,* 2011)

***In vitro* studies**

Drug diffusion studies were carried out using Diffusion cell apparatus (model EDC-07, Electro lab). Formulations were subjected for *Invitro*diffusion through dialysis membrane (0.65 µm) using Franz diffusion cell. A sample of 100mg was directly weighed onto the membrane over which a circular glass disc was placed and then covered by a stainless steel cap. The whole setup was held with a tight clamp. The receiver compartment contains 12ml of pH7.4 phosphate buffer and the magnetic stirrer rotating at 800rpm. The temperature (32 ± 0.5°C) was maintained by the water jacket. The test was carried out for 6 hrs. The sample was withdrawn at specific time intervals such as 30, 60, 120, 180, 240, 360 min from the receiver compartment and replenishing with fresh PBS solution (Sink condition). The experiment was run in three independent cells with three formulations respectively. The samples were analyzed spectrophotometrically at 260 nm. (Yerramsetty *et al.,* 2010; Friend D.R, 1992).

Formula: % Drug diffused=$\frac{Cr\times Vr}{A}\times 100$

Stability Studies

The stability study was performed as per ICH guidelines. The optimized formulations of gel were filled in the collapsible tubes and stored at different temperatures and humidity conditions, viz.250˚c ± 20˚c/ 60% ± 5% RH, 300˚c ± 20˚c/ 65% ± 5%rh, 400˚c ± 20˚c/ 75% ± 5% RH for a period of three months and samples were analyzed for appearance, pH, viscosity and spreadability (Aggarwal *et al.,* 2012).

RESULT AND DISCUSSION

Compatibility studies

Drug and excipients in 1:1 ratio were mixed and stored in glass vials at room temperature, 50°C and 60°C. The samples were analyzed for compatibility after 4 weeks. No changes were observed in the physical appearance of the mixture in 4 weeks as shown in figure 1. Hence there is no any incompatibility between drug and excipients.

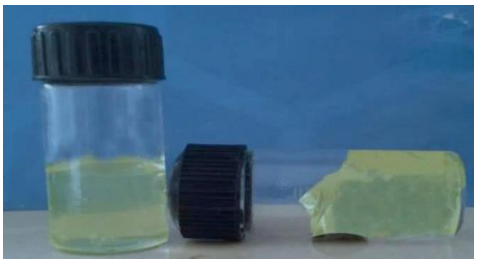

Figure 1: Physical appearance of Hydrogel

Table 2: Evaluation Studies

Batch No.	Colour & Appearance	Homo-gene-ity	pH Mean ±SD*	Viscosity (cps) Mean ± SD*	Spread-ability (cm) Mean ± SD*	Drug content (%w/w) Mean ± SD*	Content uniformity (% w/w) Mean ± SD*
F1	Yellow, Clear& translucent	Good	7.023± 0.002	2.18Lakhs cps/39SR	2.7 ± 0.05	91.77 ± 0.04	92.16±0.04
F2	Yellow, Clear& translucent	Good	7.116± 0.007	27Lakhs cps/40SR	2.3 ± 0.05	91.85 ± 0.03	92.3±0.02
F3	Yellow, Clear& translucent	Good	6.809± 0.007	3.78 Lakhs cps/42SR	1.5 ± 0.16	94.50 ± 0.01	94.75±0.03
F4	Yellow, Clear& translucent	Good	7.023± 0.002	3.69 Lakhs cps/40SR	1.3 ± 0.18	91.79 ± 0.03	92.15±0.04

Table 3: % drug release formulation

Time (hrs.)	% Drug diffused (w/w) Mean ±SD*			
	F1	F2	F3	F4
0.5	9.86 ± 0.02	9.35 ± 0.02	8.02 ± 0.01	5.71 ± 0.01
1.0	26.53 ± 0.026	25.62 ± 0.02	17.63 ± 0.01	12.02 ± 0.02
2.0	35.94 ± 0.045	30.56 ± 0.02	34.65 ± 0.02	21.04 ± 0.01
3.0	43.64 ± 0.02	41.02 ± 0.02	38.83 ± 0.01	33.41 ± 0.01
4.0	48.86 ± 0.02	48.23 ± 0.02	47.10 ± 0.08	40.21 ± 0.01
5.0	52.72 ± 0.02	52.46 ± 0.02	53.23 ± 0.03	48.22 ± 0.02
6.0	58.72 ± 0.02	57.02 ± 0.02	56.35± 0.02	55.20 ± 0.01

Table 4: Data for stability studies of formulations

Batch No.	Storage Conditions	Physical appearance	Drug content(%w/w)			
			0 Month	1st Month	2nd Month	3rd Month
F1	25°C/60%RH	No change	91.139±0.01	90.166±0.02	90.142±0.012	89.003±0.03
	30°C/65%RH	No change	91.139±0.01	91.012±0.045	90.148±0.03	90.122±0.02
	40°C/75%RH	No change	91.139±0.04	90.251±0.023	89.416±0.01	89.268±0.02
F2	25°C/60%RH	No change	91.392±0.03	91.330± 0.01	90.428±0.02	90.314±0.02
	30°C/65%RH	No change	91.392±0.02	90.275±0.03	90.138± 0.01	89.376±0.02
	40°C/75%RH	No change	91.392±0.02	89.147±0.02	89.162± 0.05	88.925±0.01
F3	25°C/60%RH	No change	93.161±0.02	93.112± 0.01	90.248±0.03	90.224±0.02
	30°C/65%RH	No change	93.161±0.01	92.886±0.02	92.314±0.02	92.078±0.03
	40°C/75%RH	No change	93.161±0.01	92.732±0.02	92.085±0.02	89.956±0.01
F4	25°C/60%RH	No change	92.151±0.02	92.00±0.03	91.249± 0.01	90.164±0.03
	30°C/65%RH	No change	92.151±0.01	91.851±0.02	90.125± 0.01	89.002±0.02
	40°C/75%RH	No change	92.151±0.01	90.329±0.03	89.32 ±0.01	88.953±0.02

Evaluation Studies

The results are shown in table 2 the appearance and the clarity of all prepared formulations was found to be yellow translucent and uniform. The pH of all formulations was found to be around pH 7.00 which is in the range of skin pH and does not cause any irritation. The viscosity of all formulations was found to be satisfactory and was in the range of 2.18lakhs cps to 3.98lakhs cps. Which indicates that as the concentration of carbopol increase the viscosity of gel also increased. The spreadability of the formulation was found in the range of 1.3cm to 2.7cm. Spreadability of the formulations decreased with increase in the concentration of carbopol, due to increase in viscosity. The drug content of all prepared formulation was within the acceptable range, (NLT 90% and NMT 105% as per USP) and ensures dose uniformity.

***In vitro* drug diffusion studies**

From the below *In-vitro* drug diffusion data in Table 3 it revealed that the drug diffusion gradually decreased from F1 to F4 due to an increase in the concentration of carbopol934 as shown in Figure 2. The carbopol retards the release of the drug from the gel.

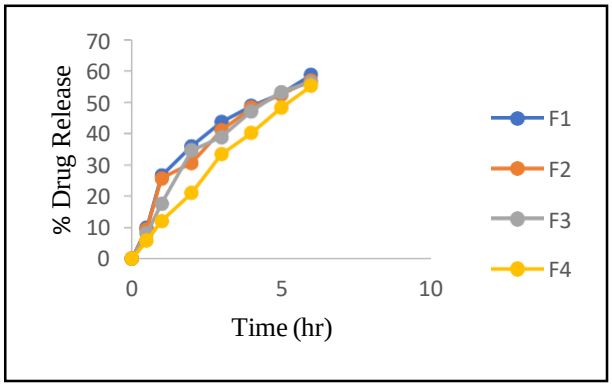

Figure 2: Comparison graph for In-vitro diffusion studies of all formulation

STABILITY STUDIES

There was no marked change in physical appearance and assay. The results are shown in Table 4 of the stability samples, indicated that the consistency remained almost the same as the initial samples.

The results of stability studies showed that there was no significant change in the appearance, pH, drug content and *in vitro* release. the formulations were found to be stable at 25˚C / 60% RH, 30˚C / 65% RH and 40˚C / 75% RH.

CONCLUSION

From the study, it can be concluded that, among all the formulations prepared, F1, F2 F3 exhibited poor spreadability and extrudability. However the former two had a higher percentage of drug diffusion, the later one showed poor diffusion. The Formulation F3 had a comparatively similar percentage of drug diffusion and also exhibited good spreadability and extrudability. As a result, it was concluded that F3 was the optimized formulation.

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