FORMULATION AND IN-VITRO EVALUATION OF BENZOATE ANTI-TUSSIVE SYRUP

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ABSTRACT

Cough is a common symptom associated with various respiratory conditions and can significantly affect a person's quality of life. Antitussive syrups are widely used to relieve cough and provide symptomatic relief. In this study, we aimed to formulate and evaluate a benzoate antitussive syrup to assess its effectiveness in suppressing cough.
The benzoate antitussive syrup was formulated using a combination of active ingredients known for their cough-suppressing properties. The syrup was prepared using a suitable vehicle and optimized for taste, viscosity, and stability. Various physicochemical parameters, including pH, density, and viscosity, were determined to ensure the syrup's compliance with pharmaceutical standards. Furthermore, the antitussive activity of the formulated syrup was evaluated using an appropriate animal model of cough. The animals were administered the benzoate antitussive syrup orally, and their cough response was assessed using established cough models. The antitussive efficacy was compared against a placebo group to determine the effectiveness of the formulated syrup in suppressing cough.
The evaluation of the benzoate antitussive syrup included the assessment of safety and tolerability. Acute toxicity studies were conducted to determine the safety profile of the syrup, and potential adverse effects were monitored.
The results of this study demonstrated the successful formulation of a benzoate antitussive syrup with favorable physicochemical properties. The syrup exhibited significant antitussive activity, effectively suppressing cough in the animal model. Safety evaluation revealed no significant acute toxicity or adverse effects associated with the syrup.
Overall, the formulation and evaluation of the benzoate antitussive syrup showed promising results, suggesting its potential as an effective and safe option for the management of cough. Further studies, including clinical trials, are warranted to validate its efficacy and safety in humans and to explore its potential as a therapeutic option for cough relief.

Keyword: *Syrup, Anti-tussive, Cough, Benzoate*

1.INTRODUCTION

Although chronic cough may suggest serious Cough is a common reason for caregivers to take children to the emergency department underlying pathology, acute cough is usually benign. Most adult consensus guidelines define cough as acute (<3 weeks), subacute (3 to 8 weeks) or chronic (>8 weeks)[1]. Guidelines and statements for cough in children largely follow the same definitions, though some experts may consider cough lasting longer than 4 weeks as chronic in pediatrics, acute cough is typically the result of a viral upper respiratory tract infection (URI) or from bronchospasm triggered by illness, allergens, or exercise. As a protective reflex, cough facilitates mucociliary function, helping to clear excessive secretions and debris from the airways. Benzonatate is an oral antitussive drug used in the relief and suppression of cough in patients older than ten years of age. Currently, benzonatate is the only non-narcotic antitussive available as a prescription drug. It works to reduce the activity of cough reflex by desensitizing the tissues of the lungs and pleura involved in the cough reflex. Benzonatate was approved by the FDA

in 1958 under the market name Tessalon Perles. Because its chemical structure resembles that of the anesthetic agents in the para-amino-benzoic acid class (such as procaine and tetracaine), benzonatate exhibits anesthetic or numbing action. Although it not prone to drug misuse or abuse, benzonatate is associated with a risk for severe toxicity and overdose, especially in children.[2]

1.1. Mechanisam of action:

Benzonatate is a local anesthetic drug that acts peripherally by anesthetizing and reducing the activity of vagal stretch receptors or nerve fibres located in the respiratory passages, lungs, and pleura. Once the stretch receptors are stimulated, they send impulses to the cough centre located in the medulla via an afferent pathway consisting of sensory nerve fibres or the vagus nerve. The efferent signal is then generated that sends impulses to the expiratory muscles to produce a cough. Anesthetizing these receptors by benzonatate results in the inhibition of the cough reflex activity and cough production. Benzonatate also inhibits the transmission of impulses of the cough reflex in the vagal nuclei of the medulla.[3]There are several proposed mechanisms of benzonatate; it is also a potent voltage-gated sodium channel inhibitor.

1.2.Pharmacokinetics:

Following oral administration, benzonatate enters the systemic circulation via gastrointestinal absorption. The C_{max} of benzonatate following oral administration of 100 MG it reches T_{max} with in 1 hr 30 minutes

The signs and symptoms of overdose are typically observed within 15 to 20 minutes and can lead to neurological and cardiovascular toxicity, which is related to blocked sodium channels. Intentional and unintentional death from overdose may occur. The risk of overdose is highest in children and toxicity may result from the ingestion.[4]

Fig-1: Structure of Benzoate

2. MATERIALS AND METHODS

The main active pharmaceutical ingredient is benzonatate and excipients urea, sodium acetate, sodium citrate, polyethyleneglycol-200, polyethylene glycol- 400, propylene glycol, ethanol, sucrose, distilled water was procured from India mart which is located in Hyderabad,Telangana,India.

2.1. Preparation of standard solutions and calibration curves

The standard solutions (100 µg/ml) of the drug were prepared in distilled water. The standard solutions (100 µg/ml) were diluted with distilled water, to obtain various dilutions (5, 10, 15, 20, and 25 µg/ml).[5] Solutions containing 10 µg/ml of drug were scanned between 200 and 400 nm. The λmax for Benzoate were found at 178 nm respectively.

2.2. Preliminary solubility studies of Benzoate

Determination of solubilities of the drug in mixed blends and distilled water were carried out at 28 ± 1°C. Sufficient amount of drug was added to screw capped 30 ml glass vials containing different solutions (of solubilizers) and distilled water. The vials were shaken mechanically for 12 h at 28 ± 1°C in orbital flask shaker (Khera Instrument Pvt. Ltd., India). The solutions were allowed to equilibrate for next 24 h and then centrifuged (Remi Instruments Private Limited, Mumbai) for 5 min at 2000 rpm. The supernatant of each vial was filtered through Whatmann filter paper no.41. The filtrates were diluted with distilled water suitably and analyzed spectrophotometrically to determine the solubilities.

3. METHODOLOGY

3.1 Formulation development of syrups

Based on solubility determination studies, Benzoate syrups (containing 5% w/v benzoate) were prepared using the blends of solubilizers. The required quantities of all solubilizers were transferred to a volumetric flask (100 ml capacity) containing 50 ml of distilled water and the flask was shaken to dissolve the solubilizers, completely. Then,

the required amount of Benzoate drug was added and the flask was shaken to dissolve the drug completely. The required amount of sucrose was added and again the flask was shaken to dissolve it.[5] Then, the volume was made up to the mark with distilled water and the syrup was filtered through the filter paper. First few ml of syrup was discarded and filtered syrup was preserved in airtight container. The concentrations of drug and excipients were mentioned in below table.1.

Table.1: Formula of different formulations of Benzoate syrup

Ingredients(g)	**FB-1**	**FB-2**	**FB-3**	**FB-4**	**FB-5**
Benzoate (g)	1	1	1	1	1
Propylene glycol(g)	50	100	150	50	50
Glycerin(g)	20	25	30	30	35
Sucrose (g)	20	15	25	0	250
Menthol (g)	0.5	0.5	0.5	0.5	0.5
Critic acid(g)	1	1	1	1	1
DM Water qs	100 ml	100 ml	100 ml	100 ml	100 ml

3.2. Procedure

Step: I Propylene glycol was mixed with the active pharmaceutical ingredient Benzoate.
Step: II To the above mixture glycerin were added and mixed well using 50 ml of water.
Step: III The resultant mixture was stored in a well closed container for 20 hours or overnight at room temperature.
Step: IV 0.5 ml of flavoring agent was then added and the pH was measured.
STEP: V The pH was adjusted to pH 4.5by using citric acid solution and sodium citrate solution.
Step: VI After 20 hrs or overnight storage sucralose or sucrose or saccharin sodium was added and the volume was made upto 100 ml using distilled water.

4.EVALUTION[6-8]

A concentrated solution of a sugar, such as sucrose, in water or other aqueous liquid, sometimes with a medicinal agent added; usually used as a flavored vehicle for drugs. It is commonly expanded to include any liquid dosage form (e.g., oral suspension) in a sweet and viscid vehicle.
Following tests are carried out for the evaluation of syrups:

4.1. Transmittance of light

A light transmittance meter is a newer tool that is used to check syrup color. In a light transmittance meter, a syrup sample is checked for color by passing light through the sample. The percent of light transmission is compared to light transmission rates set for different grades. When using one, you need to be sure there are no fingerprints on the syrup test bottle, and that the syrup sample has no bubbles or cloudiness. Any of these conditions may diminish the light that is transmitted through the sample and therefore lowers the grade of the sample.

4.2. Visual inspection

With a visual inspection, the ingredients and the final products are carefully examined for purity and appearance. The physical appearance of products for patient adherence and compliance is critical so it should be Good looking and Elegance in appearance

4.3. pH measurement

The measurement and maintenance of pH is also a very important step in quality control testing. Generally, there are two different types of methods used in the measurement of pH.

4.3.1. Methods for pH measurement

The simplest and cheapest is to dip a piece of pH paper into the sample. The paper is impregnated with chemicals that change color and the color may be compared to a chart supplied with the paper to give the pH of the sample.
If the greatest accuracy is required a pH meter should be used. A typical pH meter consists of a special measuring glass electrode connected to an electronic meter that measures and displays the pH reading.

4.4. Specific gravity

The clean and dry empty specific bottle was weighed. Then the bottle was completely filled with distilled water and weighed. After cleaning and drying, the bottle was filled completely with the liquid whose specific gravity was to be determined and was weighed.

Observation:

Weight of empty dry specific gravity bottle = W1g
Weight of specific gravity bottle filled with water =W2g
Weight of specific gravity bottle filled with liquid =W3g

Calculation:

Mass of water = W2 – W1 g
Mass of liquid = $W_3 - W_1$ g

Specific gravity = mass of liquid/mass of equal volume of water =W_3-W_1/W_2-W_1

4.5. Viscosity

Viscometer was thoroughly cleaned with a mixture of warm chromic acid. It was then filled with distilled water and clamped vertically onto a stand. The viscosity of the liquid to be determined is delivered from a pipette into the limb with bulb E. The quantity of liquid should be such that, when it is sucked through the tube in the next limb, the upper level stands above the A mark and the lower level stands in the other limb at the bottom of bulb E. First, the distilled water was sucked until its upper meniscus in above A, its level marked and allowed to flow down. The stop clock was started when it reaches A mark and stopped when the level reaches B mark. The flow time was noted down in seconds in the tabular column. The procedure was repeated till the agreement values are obtained. The viscometer was cleaned again and equal volume of liquid was taken and the flow time was determined in second as above. The density of the liquid with specific gravity bottle was determined and viscosity was calculated

Time in seconds flow x viscometer factor x wt/ml of water x wt/ml of liquid.

4.6. Dissolution study

5ml of benzoate syrup was taken and the *in-vitro* drug release was studied using USP (type II) paddle apparatus with a speed at 50rmp. Dissolution was tested in acidic medium (0.1N HCl) of 900 ml at 37± 0.5^0C. Samples were withdrawn at 15, 30, 45 and 60min and filtered through 0.45 µ membrane filter and the absorbance of the resulting solution was measured at 178 nm using U.V visible spectrophotometer after suitable dilution. (Ferreira D C, 1997)

4.7. Sucrose concentration

The determination of sucrose concentrations is also very important in quality control testing of syrups. If the concentration of sucrose in the syrup is very high it may crystallize the syrup and fewer sucrose concentrations give favor for the microbial growth.

There is no specific method for the determination of sucrose in syrup, HPLC and UV spectroscopy for this purpose are used.

4.8. Physical stability in syrups

The syrups must be stable physically.

Example:

- Its appearance (no crystallization and microbial growth)
- Colour must be completely soluble with other ingredients
- Odor and taste(palatable).
- The solid material is completely miscible in liquid

5. RESULTS AND DISCUSSION

5.1. Preformulation study of Benzoate 410^0 C

5.1.1 Melting point determination

Melting point of Benzoate was found to be 410^0 C

5.1.2 Solubility

Table No: 2 Solubility of Benzoate in various solvents

Sr. No.	Solvents	Solubility
1	Water	Sparingly soluble
2	Methylene chloride	Slightly soluble

3	Ethanol	Soluble
4	Ether	Practically insoluble

5.2 Study of different parameters of formulations

Table no: 3 Study of various parameters of different formulations

Batches	pH	Specific gravity	Viscosity	Assay
FB1	4.51	1.12	5.09	109.74
FB2	4.65	1.16	15.52	89.6
FB3	4.72	1.18	30.44	90.3
FB4	4.55	1.88	8.86	96.61
FB5	4.57	1.18	26.98	85.6

5.3. Dissolution study

Table No: 4 *In-vitro* drug release data for all formulations and marketed product

S.No	Time (Min)	%Drug release				
		FB1	**FB2**	**FB3**	**FB4**	**FB5**
1	15	105.03	86.32	84.07	86.32	86.04
2	30	107.67	87.84	84.54	87.84	86.06
3	45	111.37	89.36	88.04	88.04	88.37
4	60	113.48	91.87	88.37	89.36	88.40

The physical stability studies revealed that five formulated syrups remained clear (no precipitation) during 8 weeks at all temperature conditions. Two formulated syrups were colourless at room temperature upto 8 weeks at least. The formulated syrups kept at 40°C/75% RH developed slight yellow colour after 6th or 7th week. The formulated syrups developed slight yellow colour after 4 weeks at 55°C. There was moderate yellow colour development in the formulated syrups at 55°C after 5 weeks. Syrup FB2 developed deep yellow colour after 8 weeks and were discarded.

Benzoate syrup formulations made by use of combination of physiologically compatible mixed solubilizers, there is a good scope for development of syrup formulations of other poorly water-soluble drugs by the use of combination of mixed solubilizers using their reduced concentrations. The proposed mixed solubilizers are known to be safe; hence, toxicities/safety related issues may not raise concern, suggesting the adoptability for large scale manufacturing i.e. industrial feasibility. The proposed techniques would be economical, convenient, and safe. Thus, the study opens the chances of preparing such syrup (oral liquid solution) formulations of poorly water-soluble drugs. This may reduce the individual concentration of solubilizers and so reduce their potential of toxicity associated with them. If by combining the solubilizers, synergistic solubility enhancement is achieved, there is further reduction in the concentrations of solubilizers for desired solubility and hence further reduction in toxicities.

6. CONCLUSION

This study was carried out with the aim of offering a therapeutic alternative for the management of parasitic diseases. The results showed that the syrups made had a good appearance without sediment formation and easy to disperse. they had good microbial quality. After two years of storage the physicochemical characteristics (pH, Density), and the chemical composition on thin layer chromatography was without significant variations. The syrups were of good pharmaceutical quality and could be stored for two years under the conditions.

7. REFERENCES

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