Diagnosis and Treatment of Prosthetic Joint Infection (PJI)

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PROSTHETIC JOINT INFECTION

Total knee arthroplasty (TKA) also known as total knee replacement (TKR) is a surgical procedure in which damaged parts of the knee joint are replaced with metal typically (cobalt–chromium or titanium), and plastic (an ultrahigh-molecular-weight polyethylene material).

The surgery is done by separating the muscles and ligaments around the knee to expose the inside of the joint. The ends of the thigh bone (femur) and the shin bone (tibia) are removed as is often the underside of the kneecap (patella). The artificial parts are then cemented into place using poly methyl methacrylate (PMMA) cement.

Although prosthetic joint implantations improve patients' quality of life, these procedures are associated with complications, including aseptic failure (i.e., aseptic loosening) and prosthetic joint infection (PJI). More than 25% of all prostheses will eventually demonstrate evidence of loosening, often necessitating a revision arthroplasty.[1] Infections associated with prosthetic joints occur less frequently than aseptic failures, but represent the most devastating complication with high morbidity and substantial cost. In addition to protracted hospitalization, patients risk complications associated with additional surgery and antimicrobial treatment, as well the possibility of renewed disability[2]

In patients with primary joint replacement, the infection rate in the first 2 years is usually <1% in hip and shoulder prostheses, <2% in knee prostheses, and <9% in elbow prostheses. The reported infection rates are probably underestimated, since many cases of presumed aseptic failure may be due to unrecognized infection. In addition, infection rates after surgical revision are usually considerably higher (up to 40%) than after primary replacement.[1]

Treatment of an infected prosthetic joint usually exceeds the conservative estimate of $ 50000 per episode[2]. Importantly, prosthetic joints remain susceptible to haematogenous seeding during their entire lifetime and some perioperative infections may have a latency period longer than two years[2]. Risk factors include Immunosuppression, Previous joint arthroplasty, Malignancy, Steroid therapy,Bacteraemia,Revision of existing prosthetic joint,Diabetes mellitus, aging, obesity, poor nutrition, skin disease, rheumatoid arthritis, osteoarthritis. PJI can be classified based on-

- According to onset of symptoms

Early (Withing 3 Months of Implantation)	Delayed (3-12 Months After Implantation)	Late (More than 12 Months After Implantation)
• Organisms acquired at the time of surgery. • Presents as continous leaking wound, acuteonset of fever, swelling, effussion, erythema, large hematoma, pain at implant site	• Organisms acquired at the time of surgery • increasing joint pain, early loosening are hall marks of delayed infection. • clinical signs of infection may be absent.	• Haematologically acquired infection • Presents as worst joint pain, effusion, restriction of movement, sinuses also occur. • unrecognised bacteraemia

- According to route of infection

PERIOPERATIVE - inoculation of microorganisms into the surgical wound during surgery or immediately or there after

HEMATOGENOUS - from a distant foci of infection it spreads through blood or lymph.

CONTIGUOUS - contiguous spread from an adjacent focus of infection (eg, penetrating trauma, preexisting ostemyelitius, skin and soft tissue infections.

Pathogenesis of PJI can be described as -

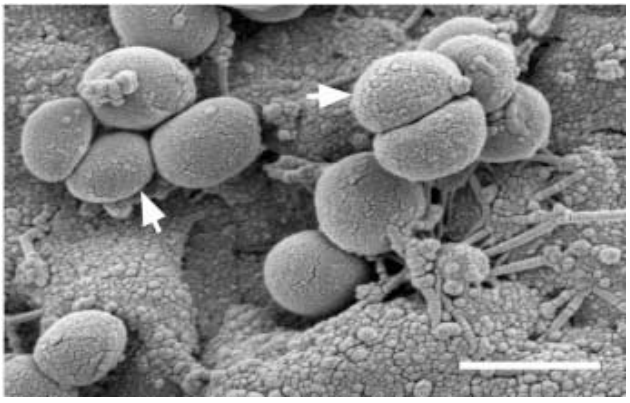

FIG 1- STAPHYLOCOCCUS AUREUS BIOFILM

Microorganisms live in clustered form in hydrated extracellular matrix (SLIME) attached to the surface. Theythrive using metabolic substances/ waste products in biofilms. Biofilms has interstitial voids called water channels through which water circulates between cells. These water channels acts as Rudimentary circulatory system. Here, Bacteria develop into complex, organized communities with functional and structural heterogeneity. Quorum sensing induces changes in bacteria gene expression. Proximity of cells within micro colony provides an environment for exchange of genes located on extra chromosomal DNA (plasmids).Thus they have a survival mechanism which helps these microbes resist against external/ internal environmental factors.

The diagnosis of PJI is difficult, because symptoms, signs, and investigations will all be non-specific. However, prompt recognition and diagnosis of PJI facilitates timely intervention to salvage infected joints, preserve joint function, prevent morbidity, and reduce costs[3].

Blood tests -UK guidelines recommend baseline blood tests for inflammatory markers (C reactive protein, erythrocyte sedimentation rate, leucocyte count) for any case of septic arthritis. However, these parameters are raised for up to two weeks after orthopaedic surgery so are non-specific for early infection[3]

Joint fluid aspiration-The combination of serology and the aspiration can help the clinician confirm or refute diagnosis of PJI [4]. It is recommended that three to five samples from various locations around the prosthesis be taken to increase the likelihood of obtaining positive culture[4]. Prior antibiotic use, slow growing organisms, and presence of biofilms are some of the factors negatively influence sensitivity of culture results. Repeating the joint fluid aspiration is a reasonable approach in patients with abnormal ESR and/or CRP in whom synovial fluid analysis is negative in the first attempt of synovial fluid aspiration.

Ultrasonography may detect fluid effusions around the prosthesis and can be used to guide joint aspiration and drainage procedures. It is especially helpful in prosthetic hip infection [1]

Nuclear medicine Bonescintigraphy with 99mTc has an excellent sensitivity, but a low specificity for diagnosing prosthetic joint infection [1]

Computed tomography (CT) and magnetic resonance imaging (MRI) CT is more sensitive than Plain radiography in the imaging of joint space. In addition, it may assist in guiding joint aspiration and selecting the surgical approach. MRI displays greater resolution for soft tissue abnormalities than CT or radiography and greater anatomical detail than radionuclide scans. The main disadvantages of CT and MRI are imaging interferences in the vicinity of metal implants.

Positron emission tomography (PET) needs further evaluation for implant imaging.

Irrigation and debridement with retention Outcomes are best in those patients with a short duration of symptoms, a well-fixed and functional implant and ideally with well-characterized microbiology demonstrating a highly susceptible organism[2]. This was largely in part due to the idea that with acute infection, bacteria had not yet formed an impenetrable glycocalyx biofilm layer along the prosthetic components. Theoretically then, by undertaking and I and D, one could diminish the bacterial load in the joint and retain fixed implants, thereby minimizing patient morbidity [4].

One –stage (direct) exchange includes the removal and implantation of a new prosthesis during the same surgical procedure. This approach is suitable for patients with intact or only slightly compromised soft tissues .If resistant or difficult-to-treat microorganisms are causing the infection, such as methicillin-resistant S. aureus (MRSA), small-colony variants of staphylococci, enterococci, quinolone-resistant Pseudomonas aeruginosa or fungi, a two-stage revision is preferred[4].

Two stage arthroplasty The first stage involves complete resection of all foreign material, debridement of surrounding infected soft tissues, and placement of an antibiotic impregnated cement spacer. The second stage involves removal of the spacer and any additional necrotic tissues, thorough irrigation, and placement of new prosthetic implants[4]. They allow effective debridement and the option of local antibiotic delivery by drug-eluting cement spacers.

Antimicrobial-loaded PMMA spacers Broadly speaking, there are two different types of spacers used during two-stage arthroplasty exchanges. Static spacers, also known as nonarticulating or block spacers and articulating spacers.The first of these articulating spacers to become popular was the “prosthesis with antibiotic-loaded acrylic cement” (PROSTALAC) spacer for the hip. Advantages of articulating over static spacer are thatfirst, both articulating and static spacers provide mechanical support during the time in which the arthroplasty is removed. This preserves proper joint position, prevents muscle contractures, and enhances patient comfort between the first and second stages. However complications include bone loss with static spacers and extensor mechanism damage and wound dehiscencewith articulating spacers[5].The significant disparity in cost compared to their cheaper static counterpart (approximately $500 vs. $3500) has prevented articulating spacers from coming into universal favor from joint surgeons[4].

The second function is to provide local antimicrobial therapy to augment systemic therapy during the time between the first and second stages. While other antimicrobials, including_-lactams, macrolides, amphotericin B, and fluconazole, have been used in selected situations, vancomycin in combination with an aminoglycoside is most commonly used. The amounts of antimicrobials mixed in 40 g of PMMA are typically 1 to 3 g of vancomycin and 1.2 to 4.8 g of gentamicin or tobramycin . It is generally accepted that spacers should use a high dose of antimicrobials, defined as at least 3.6 g of antimicrobials per 40 g of PMMA, compared to_1 g for low-dose PMMA used for prosthesis fixation[5].

Table 1-Treatment of PJI associated infection caused by common microorganism

Microorganism	Antimicrobial Agent	Dose	Route
Staphylococcus aureus or coagulase negative staphylococci Methicillin-susceptible	Nafcillin or floxacillin plus Rifampin for 2 wk. followed by Rifampin plus Ciprofloxacin or Levofloxacin	2g every 6hr 450 mg every 12hr 450 mg every 12hr 750 mg every 12hr 750 mg every 24hrs to 500 mg every 12hr	IV PO or IV PO PO PO

Methicillin resistant	Vancomycin plus rifampin for 2 wk. followed by rifampin+ ciprofloxacin± or levofloxacin± or fuscidic acid or trimethoprim- sulphamethoxazole or minocycline	1g every 12hr 450mg every 12hr 450mg every 12hr 750mg every 12hr 750mg every 24hr to 500mg every 12hr 500mg every 8hr 1DS tablet every 8hr 100mg every 12hr	IV PO OR IV PO PO PO PO PO PO
Streptococcus species (except Streptococcus agalactiae)	Penicillin G or ceftriaxone for 4 wk, followed by amoxicillin	5million U every 6hr 2g every 24hr 750-1000 mg every 8hr	IV IV PO
Enterococcus species (penicillin- susceptible) and streptococcus agalaciae	Penicillin G or Ampicillin or amoxicillin plus Aminoglycoside for 2-4 wk. followed by Amoxicillin	5million U every 6hr 2g every 4-6 hr 750-1000mg every8hr	IV IV PO
Enterobacteriaceae (quinolone-susceptible) Nonfermenters (e.g., Pseudomonas aeruginosa)	Ciprofloxacin Ceftazidime or cefepime plus Aminoglycoside for 2wk, followed by Ciprofloxacin	750 mg every 12 hr 2g ever 8 hr 750 mg every 12 hr	PO IV PO
Anaerobes	Clindamycin for 2-4 wk., followed by Clindamycin	600mg every 6-8 hr 300 mg every 6 hr	IV PO
Mixed infections (without methicillin resistant staphylococci)	Amoxicillin-clavulanic acid or Ampicillin – sulbactam or Carbapenem for 2-4 wk, followed by individual regimens according to antimicrobial susceptibility	2.2g every 8hr 3g every 6 hr According to compound	IV IV IV

*PO –oral administration, IV- intravenous administration, IM-intramuscular administration, DS- double strength (trimethoprim-160mg and sulphamethoxazole-800mg). Total duration of antimicrobial treatment with implant retention or one stage exchange should be 3 months for hip prosthesis and 6months for knee prosthesis.

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Formulation and Evaluation of Cineole Buccal Strips

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ABSTRACT

The objective of this study was to prepare Buccal strip of Cineole and to evaluate its effective release through buccal cavity using natural bioadhesive polymer. Cineole loaded buccal strips were prepared by solvent casting technique using Pectin Sodium alginate and Gelatin as polymers in three different ratios. The FTIR studies indicated that there is no incompatibility between drug and excipients. Amongst nine formulations P2 (Pectin), S5 (Sodium alginate) and G8 (Gelatin) were selected based on folding endurance and optimal tensile strength. Glycerine was used as plasticizer to produce flexible strip without having major influence on their release property. All batches were subjected to evaluations for Percentage Moisture uptake, Percentage Moisture content, Thickness, Folding Endurance, Percentage Drug content, Percentage Elongation, Tensile strength, and Adhesive strength. No significant difference in drug content was observed between the strips among the nine formulations. The in vitro release profile of formulations P2, S5 and G8 were found to be 98.41%, 78.36% and 99.41% respectively at the end of 12 h. The release kinetics confirms that the formulation S5 followed zero order, non-fickian diffusion model. The ex vivo studies results showed 66.86% of drug release at the end of 12 h. It concluded the sustaining release property of polymer. Stability study revealed that there is no significant change from its initial nature till the period of three months at 40˚C ±2˚C/75±5% RH.

Key words: *Buccoadhesive drug delivery, Gingivitis, Cineole, Buccal strip*

INTRODUCTION

Buccal delivery involves the administration of the desired drug through the buccal mucosal membrane lining of the oral cavity. The mucosal lining of the oral cavity offers some distinct advantages. It is richly vascularized and more accessible for the administration and removal of a dosage form. Additionally, buccal drug delivery has a high patient acceptability compared to other non-oral routes of drug administration[1].

Gingivitis is a form of periodontal disease. Gingivitis is due to the long-term effects of plaque deposits on the teeth. Plaque is a sticky material made of bacteria, mucus, and food debris that develops on the exposed parts of the teeth. It is major cause tooth decay. There is a clear association between gum disease and heart disease.

A 2004 study found that 91 percent of patients with cardiovascular disease also suffered from moderate to severe periodontal disease. Cineole from the essential oil of Eucalyptus globulus acts against antibiotic-susceptible and antibiotic-resistant pathogens[2].

The main aim of the study was to design and evaluate buccal strips of Cineole that provides reasonably constant effective levels of drug within the buccal cavity and offers better management of gingivitis while avoiding the adverse effects which may be associated with the conventional dosage forms. Cineole was selected based

on the literature survey. It is naturally occurring and more effective in the treatment of Gingivitis[3].

MATERIALS

Cineole was procured from Alfaesar, Lancs UK., Potassium dihydrogen ortho phosphate and Sodium hydroxide were purchased from Spectrum Reagents and Chemicals, Cochin. Pectin, Gelatin, Sodium alginate and Glycerin were purchased from Hi Pure Fine Chem Industries, Chennai.

METHODS

Preparation of Buccal Strip of Cineole

Cineole buccal strip was prepared by solvent casting method. The film was prepared in a petriplate had a diameter of 7.6 cm with 6ml capacity. The polymeric solutions were prepared using water. The polymeric solution was added to the drug cineole. Glycerin was mixed and stirred well to get a homogenous solution. The resulted uniform solution was casted on the petriplate and air dried for 24 hours. After 24 hrs the dried films were taken out, cut into 2X2cm2 and stored in desiccators for further studies. The buccal strips of Cineole with polymers and plasticizer in different ratios and combination are represented in Table 1. The quantity of drug was kept constant for all formulations[4].

Evaluation of the Buccal Strips 5,6,7,8,9

The prepared buccal strips were evaluated for different parameters such as Uniformity of weight, Thickness, Drug content, Folding Endurance, Percentage Moisture uptake, Percentage Moisture content, Surface pH, Percent Elongation, Tensile strength, Adhesive strength, Ex vivo buccal permeation study, In-vitro drug release and Stability studies. The details are depicted in Table 1 to Table 5 and Figure 1 and Figure 2.

Table No.1. Composition of formulations

S. No	Ingredients	Pectin			Sodium alginate			Gelatin		
		P_1	P_2	P_3	S_4	S_5	S_6	G_7	G_8	G_9
1	Cineole (mg)	100	100	100	100	100	100	100	100	100
2	Polymer (mg)	300	400	500	300	400	500	300	400	500
3	Distilled water (ml)	5	5	5	5	5	5	5	5	5
4	Glycerine (ml)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15

Table No. 2. Physicochemical Evaluations

Formulation code		Uniformity of weight (g)	Thickness (mm)	%Drug content	Folding Endurance (no's)	% MU	%MC	Surface pH determination	% Elongation (%mm)	TS (Kg/ mm2)	Adhesive Strength
Pectin (P)	P1	0.23±0.73	0.17 ±0.52	95.47±0.05	260 ±0.06	2.06 ±0.54	0.572± 0.60	6.4 ±0.05	66 ±0.52	3.410±0.05	10.0±0.63
	P2	0.37 ±0.1	0.23 ±0.47	95.82±0.65	270±0.57	2.14 ±0.08	1.925± 0.4	7.2 ±0.10	85 ±0.43	5.400±0.65	26.3±0.83
	P3	0.41 ±0.45	0.29 ±0.61	95.70±0.68	240 ±0.82	1.97 ±1.03	1.642±0.05	6.2 ±0.72	78 ±1.21	6.461±0.83	21.66±0.15
Sodium alginate (S)	S4	0.26 ±0.20	0.32 ±0.83	96.07±0.29	259 ±0.41	2.76 ±0.67	2.967 ±0.03	7.2 ±0.43	125 ±0.72	6.461±0.46	19.63±0.83
	S5	0.37 ±0.01	0.35 ±0.15	96.24±0.65	285±0.57	3.57±0.07	3.271±0.4	7.4 ±0.11	130 ±0.21	7.660±0.21	27.3±0.29
	S6	0.17 ±0.25	0.35 ±0.59	95.93±0.81	245 ±0.64	2.95 ±0.34	3.431±0.62	7.2 ±0.9	118 ±0.92	5.410±0.72	14.12±1.04
Gelatin (G)	G7	0.28±0.61	0.11 ±0.51	96.00±1.04	237 ±0.38	0.96 ±0.41	1.481±0.45	7 ±0.65	98 ±0.06	3.800±0.20	10.0±0.25
	G8	0.25 ±0.2	0.18 ±0.32	96.01±0.47	267± 1.0	1.88±0.19	0.192±0.08	6.6 ±0.30	113 ±0.89	4.660±0.89	22.3±0.82
	G9	0.31 ±1.26	0.20 ±0.45	95.76±0.70	245 ±0.92	1.89±0.02	1.923 ±1.01	6.6 ±0.27	86 ±0.79	6.410±1.63	26.89±0.37

MU-Moisture uptake, MC-Moisture content, TS- Tensile Strength

Mean ± S.D: n=3

Table No. 3. Comparative in vitro drug release profile

S. No	P2	S5	G8
0	0	0	0
5	0.51 ± 0.23	0.31± 0.07	0.71±0.64
10	0.95 ± 0.45	0.81± 0.64	1.34±0.52
15	1.80 ± 0.44	0.95± 0.27	1.96±0.62
30	2.54 ± 0.76	2.14± 0.05	3.27±0.09
45	5.62 ±1.78	4.32±0.73	6.34±0.62
60	9.00 ± 0.59	8.00±0.16	10.53±0.17
120	18.31±1.23	16.31±1.2	19.31±0.06
180	24.48 ± 0.61	22.12±0.05	25.56±0.06
240	32.56±1.24	30.00±0.59	33.71±0.41
300	38.73±1.90	36.41±0.01	39.46±0.84
360	48.00± 0.23	45.64±0.48	50.95±0.41
420	56.64±1.34	50.00±0.12	58.00±0.06
480	62.91± 0.91	56.51±0.73	64.62±0.92
540	70.36±0.61	66.46±0.89	74.96±0.68
600	80.31±0.72	75.56±0.51	82.31±0.6
660	90.76±1.2	76.16±0.51	93.76±0.83
720	98.41±0.56	78.36±0.08	99.41±0.53

Mean ± S.D: n=3

Table No. 4. Ex vivo buccal skin permeation study of S5 formulation

Time in min	% Drug release
0	0
5	0.21±0.15
10	0.45±0.53
15	0.67±0.71
30	1.31±0.62
45	2.12±0.41
60	2.81±0.47
120	5.32±0.16
180	10.34±0.32
240	16.32±0.06
300	22.67±0.83
360	28.56±0.71
420	34.78±0.92
480	40.56±0.63
540	46.35±0.68
600	53.56±0.73
660	59.23±0.63
720	66.86±0.58

Mean ± S.D: n=3

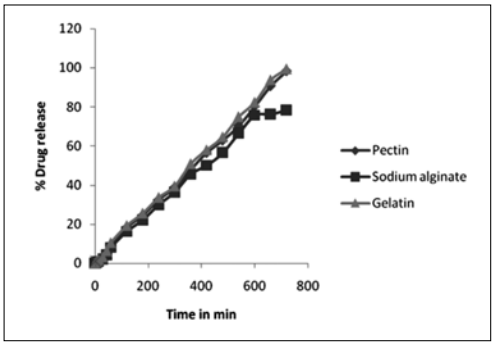
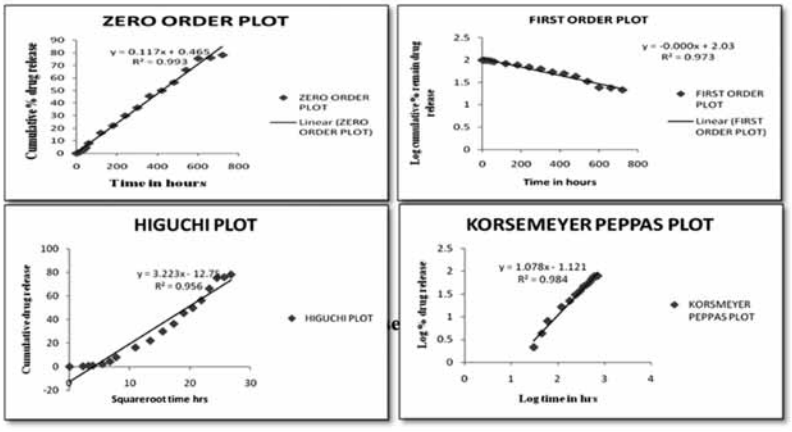


Fig 1. Comparative in vitro Release Profile of P2, S5, G8



Uniformity of weight

Each strip was weighed individually and the average weights of five were calculated using Digital balance.

Thickness

The thickness of the strip was measured using a screw gauge at different places and average thickness of five strips was reported.

Drug content

Buccal strips were cut into small pieces and dissolved in PBS pH 7.4 and measured the absorbance at 240 nm. An average

content of the strips were determined from the standard graph.

Folding Endurance

Determined by repeatedly folding one strip at the same place till it broke. The number of times the strip could be folded at the same place without breaking gave the value of folding endurance.

Percentage Moisture uptake

The Percent moisture absorption test was carried out to check the physical stability or integrity of the strip at moist condition. Buccal strips were weighed and placed in a dessicator containing 100ml of saturated solution of Aluminium chloride which maintains 79.5% humidity. After 3 days the strips were taken out and reweighed. The percentage moisture absorption was calculated using the following formula.

$$\text{Percent moisture uptake} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Percentage Moisture content

The strips were weighed and placed in a dessicator containing fused anhydrous Calcium chloride. After 3 days the strips were taken out and reweighed. The percentage moisture absorption was calculated using the following formula.

$$\text{Percentage moisture content} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Determination of surface pH

The strips were allowed to swell by keeping them in contact with 1 ml of distilled water for 2 h at room temperature and pH was noted down by bringing the electrode in contact with the surface of the strip, allowing it to equilibrate for 1 min.

Percent Elongation

When stress is applied, a strip sample stretches and this is referred to as strain. Strain is basically the deformation of strip divided by original dimension of the sample. Generally elongation of strip increases as the plasticizer content increases. It was calculated by using following formula.

$$\text{Percentage elongation} = \frac{\text{Increase in length of strip}}{\text{Initial length of strip}}$$

Tensile strength

Tensile strength is the maximum stress applied to a point at which the strip specimen breaks. It was calculated by the applied load at rupture divided by the cross-sectional area of the strip as given in the equation below:

$$\text{Tensile Strength} = \frac{\text{Load at failure} \times 100}{\text{Strip thickness} \times \text{Strip width}}$$

Adhesive strength

The adhesive strength was determined using a modified balance method. Fresh goat buccal mucosa was obtained from a local slaughter house and used within 2 hours of slaughter. The mucosal membrane was separated by removing underlying fat and loose tissues. The membrane was washed with distilled water. The goat buccal mucosa was cut into pieces and washed with phosphate buffer pH 7.4. A piece of buccal mucosa was tied to the glass vial. The glass vial was fixed tightly into a glass beaker (filled with phosphate buffer pH 7.4, at 37 ± 1˚C). So that it just touched the mucosal surface. The buccal strip was stuck to the lower side of the wooden block using a keeping a 5gm weight on the right hand pan. The 5gm weight was removed from the right hand pan, which lowered the pan along with the strip over the mucosa. The balance was kept in this position for 3 minutes contact time. Then weights were added on the right hand pan until the strip just detach from the mucus membrane. The total weight minus 5gm was taken as the buccoadhsive strength of the strip.

In-vitro drug release

Commercial semi permeable membrane was employed in this study. The Cineole strip (S_5) (2X2 cm^2) was placed in semi permeable membrane was tied to one end of open ended cylinder which acts as donor compartment. Then immersed in the receptor compartment containing 400ml of PBS pH7.4. Which was stirred at medium speed and maintained at 37°C±2°C. Samples were withdrawn at regular time intervals and the same volume was replaced by fresh diffusion medium7. The samples were analyzed using UV – visible spectrophotometer (Shimadzu UV1700) at 240 nm.

Ex vivo buccal permeation study of Selected (S_5) formulation

Ex vivo buccal permeation studies were carried out using Goat buccal skin. The receptor compartment consisted of 400ml of Phosphate buffer (pH 7.4) in 500 ml beaker. Temperature was maintained at 37 ± 0.5°C and stirred at 900 rpm. The Cineole strip (S_5) (2X2 cm^2) was placed in Goat buccal skin and tied to the one end of open-ended glass cylinder that was then dipped into freshly prepared phosphate buffer on magnetic stirrer. 5ml of samples were taken from receptor medium at various time intervals and the same volume of medium was replaced with fresh buffer. All the Samples were analyzed spectrophotometrically at 240nm using PBS 7.4 pH as blank.

Stability study

Stability studies was carried out by exposing buccal strips (S5) at Room temperature and 40˚C ±2˚C/75±5% RH and were analyzed for their Physical appearance, Colour, Texture and Percentage Drug content at the end of 1st, 2nd and 3rd months.

RESULTS AND DISCUSSION10

In the present investigation, efforts were made to prepare Buccal strips of Cineole using various polymers such as Pectin, Sodium Alginate and Gelatin in various proportions and combinations.

Nine batches (P_1, P_2, P_3, S_4, S_5, S_6, G_7, G_8, and G_9) of Cineole loaded buccal strips were prepared. One formulation from each (i.e., P_2, S_5, and G_8) was selected based on physico chemical parameters.

Among these three batches, S_5 showed maximum Thickness (0.35mm), folding endurance (265), Percent Drug content (96.24%) and Percent Elongation (97%). The amount of polymer influences folding endurance. If the quantity was less than 0.4g handling of the strip was very difficult and if the quantity was greater than 0.4g, the strip became thick or break with insignificant tensile strength.

Trial batches P_2 and G_8 showed more than 98% of drug release at the end of 12 h. These two trial batches were not satisfied the objective of release of drug more than 12 h. A required quantity of 78.36± 0.08 of drug was released from S_5 at the end of 12 h. It revealed that 21.64% of drug was retained and it could be released up to 20 hrs. Hence formulation S_5 has been proved to be of great interest and fitted with various kinetics equations and calculated regression coefficient R2, Slope & 'n' value. From the graphical representation it could be understood that S_5 followed zero order kinetics which had shown a regression coefficient (R^2) of 0.993 and Higuchi model shown the regression value 0.956 suggested that the release of the drug by swellable polymer matrix through the diffusion of buccal fluids. Korsemeyer - Peppas equation was used to analyze the release pattern of the drug from the polymeric system. The "n" values of formulation S_5 indicated that the release followed Non-Fickian diffusion mechanism. The ex vivo study results showed 66.86% of drug release at the end of 12 h. It concluded the possibility of sustained release property of polymer through buccal mucosa of goat.

The stability study of S_5 at Room temperature and 40˚C ±2˚C/75±5% RH showed no significant changes in Visual appearance, Colour, Texture and Percentage Drug content. It confirmed that the prepared buccal strips were stable for three months.

CONCLUSION

The formulation S5 provides reasonably constant effective levels of drug within the buccal pH for a period of 12 h. The kinetic data of in vitro dissolution indicated that the Buccal strip (S5) followed Non-Fickian diffusion kinetics. Also ex-vivo studies confirm that the effect of buccal strips, containing 100 mg of Cineole on goat buccal mucosa showed prolonged release for 12 h. The physico chemical parameters of buccal strips prepared by using Sodium alginate in the ratio of 1: 4 showed good results so, it can be predicted that there is no change in stability of the buccal strips at or below 37oC.

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Development of Topical Drug Delivery For Nabumetone Solid Dispersion

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ABSTRACT

Nabumetone (NBT) is BCS class II drug with low solubility. Its dose is 750 mg and treats discomfort caused due to arthritis. Formation of solid dispersion of NBT with carriers like urea will enhance the bioavailability. Phase-solubility studies revealed AL type of curves showed that the dispersion of urea with NBT significantly increases solubility of drug. The dispersions of NBT were carried out by different methods and evaluated for in vitro drug release, drug content, FTIR, DSC, XRD. All dispersions showed improvement in dissolution rate in comparison with pure drug. These evaluation techniques showed distinct loss of drug crystallinity and showed improvement in dissolution rate. All dispersions were found stable after stability study. Methods showing best drug release were selected for further study of development and evaluation of topical gel formulation. A topical gel has been developed using carbapol 940, propylene glycol, sodium lauryl sulphate. The formulations were evaluated for the physico-chemical and release characteristics. The optimized batch of gels showed good mechanical and physicochemical properties. The results indicated that gel with good bioadhesive and permeability properties could be prepared. The in vitro diffusion study showed drug release with urea was 76.49% in distilled water with solvent wetting method after 8 hrs.

Key words: *Solid dispersion, Nabumetone, Urea, Topical , Gel, Urea*

INTRODUCTION

BCS class II i.e. poorly water-soluble drugs often show low bioavailability when administered orally. The absorption of drugs in the GI tract is a rate-limiting step and this result in variations of dissolution rate and incomplete bioavailability. The challenging and important step in the process of drug development is improvement of the dissolution rates and bioavailability of water-insoluble drugs. Chemically, Nabumetone (NBT) chemically is 4-(6-methoxy-2-napthyl) 2-butanone. It has half life of 23 hours & dose is 750 mg. It is used for the relief of pain and discomfort occurred because of arthritis. NBT is soluble in methanol, ethanol & isopropanol. It is almost insoluble in water as reported solubility in water is less than 0.00193 mg/ml. It is necessary to improve the dissolution rate of NBT to enhance the bioavailability. There are many chemical or formulation approaches for improvement in drug dissolution and bioavailability. For improving the dissolution and bioavailability of poorly soluble drug most successful technique is solid dispersion among the various techniques. There are various methods for preparation of solid dispersion. All methods are simple, economic, and advantageous to enhance dissolution rate. (1,2,3,4)

Skin is one of the most easily available routes for drug administration and main route for topical drug delivery system. Topical preparations are applied on the skin for local or systemic effects. Topical drug delivery can be defined as the application of formulations containing a drug to the skin for treatment of cutaneous disorder. Topical delivery is an attractive route for local and systemic treatment. Gels are relatively newer class

of dosage forms created by entrapment of liquid in a network of colloidal particles. These particles may consist of inorganic or organic polymers of natural or synthetic origin. Appearance of gel depends upon the nature of colloidal substances and liquid in the formulation. (5, 6)

Topical gels are semisolid formulations consist of a high ratio of solvent/gelling agent. Gels are transparent or translucent in appearance with high degree of physical or chemical cross-linking. On dispersing gelling agent in solvent it forms colloidal network structure and limits fluid flow as solvent molecules get entrapped in the colloidal network. Gels have viscoelastic property. The matrix structure formed during storage which breaks easily on shaking or squeezing. It is easy to apply on skin as gets thinner on pressure and adhere to the skin after application. This it has better application property and stability in comparison to other topical preparation. Topical gels are intended for skin application for local action or penetration of medicament or for their emollient or protective action. The gels are non-greasy and can be washed easily. (7, 8, 9, 10)

MATERIALS AND METHODS

Materials

Nabumetone was supplied as a gift sample by Triveni Chemicals, Vapi, India. Urea were gifted from Analab fine chemicals, Mumbai, India. Carbapol 940, sodium lauryl sulphate, ethanol and triethanolamine were also gifted from Analab fine chemicals, Mumbai, India.

Methods

Drug Characterization

Melting point of NBT was determined by capillary method with use of melting point apparatus to assess purity of NBT. From the calibration curve of NBT on UV (Varian Carry 100, Australia), λmax was selected.

Solubility

NBT solubility studies were performed by adding excess amounts of NBT in water and flasks were kept in shaker for 48 hrs. The concentrations were calculated by analyzing absorbances of solutions on UV.

Infra Red Spectroscopy

To characterize NBT, FTIR (Varian 640 IR, Australia) was used. The samples were prepared by the KBr pellet method and spectra were scanned over IR frequency range.

Phase Solubility Studies

An excess amount of NBT was added to aqueous solutions of urea in increasing concentration (1%, 2%, 3%, 4% and 5%w/v). The flasks were sealed and kept in mechanical shaker at 37±0.5ºC for 72 hrs. Aliquots were withdrawn, centrifuged, filtered, suitably diluted and absorbances measured on UV at 228nm. Solubilities of NBT at different concentrations of urea were calculated.

Preparation of SD

SDs was prepared at 1:1, 1:2, 1:3 ratios, by following methods.

Physical Mixtures (PM)

The NBT with urea was grinded thoroughly in a mortar, sieved.

Solvent Wetting Method (SW)

The NBT was dissolved in methanol, kept in sonicator for 15 minutes and this solution was then dropped into carriers placed in mortor and constantly stirred. The solvent was evaporated at room temperature in desiccator.

Saturation Solubility

By keeping equilibrium of an excess amount of NBT and SDs in 10 ml distilled water kept on a mechanical shaker at room temperature for 48 hrs. Then aliquots were withdrawn, centrifuged, filtered, suitably diluted and analyzed by UV at 228 nm to determine concentration of NBT.

Characterization of SD

Percent Drug Content and Yield Study

The SD equivalent to 100 mg/ml of NBT was added in 5ml methanol, sonicated for 10 min., volume was adjusted suitably with distilled water. The solution was filtered, suitably diluted and assayed on UV at 228 nm.

The NBT content was calculated by using calibration curve. The SD was weighed and yield was calculated by using formula as

% Yield = (a/b) X 100

'a'= practical weight of SD obtained and 'b'= theoretical weight of SD.

In Vitro Release Study

In vitro release studies of NBT from SD were studied in conditions as dissolution medium used was 900 ml distilled water, 37±0.5ºC temperature and 75 rpm. The apparatus used was USP I dissolution test apparatus, basket type (make: TDT-08L Electrolab, Mumbai, India). Samples were withdrawn at specified time interval, filtered, diluted suitably and assayed for concentration of NBT using UV at 228 nm. Experiment performed in triplicates. Dissolution profiles of SDs were analyzed by plotting graph of time versus % drug release.

Fourier Transform Infrared Spectroscopy Study (FTIR)

The spectra of NBT, carriers and SDs were scanned in the range of 4000 to 500 cm-1 and recorded with FTIR spectrophotometer using KBr pellets. The drug-carrier interactions were studied from these spectra.

Powder X-Ray Diffraction Study (PXRD)

XRD with make Philips PW 1729, Netherlands was used for analyzing XRD patterns of NBT and SDs with conditions as Ni filter, CuK radiation, 20 mA current and 0.2 inch receiving slit. The samples were analyzed in range of 5° to 50°, with 2θ scan step size of and 1 second scan step time.

Differential Scanning Calorimetry Study (DSC)

DSC (Schimadzu Corporation, Star 821 e, Switzerland) was used to analyze the curves of NBT, carriers and SDs representing the rates of heat uptake. DSC was calibrated prior to analysis using an indium standard. About 2-5mg of sample was weighed in a standard open aluminum pans with conditions as 40-250°C scanning range, 10°C/min heating rate and purged with dry nitrogen.

Stability Study of SDs

The selected SDs were packed in tightly closed bottles which were capped with aluminum. They were stored at different relative humidity (RH) levels as 250C/60% RH, 300C/65% RH, & 400C/75% RH for 3 months. These SDs were evaluated for their physical changes such as color and texture, drug polymer interaction using FTIR, drug content and In vitro drug release study.

Formulation of topical gel

SD showing high solubility was selected for further development of topical gel. Gel formulations were prepared by dispersing 5% w/w Carbapol 940, 1% w/w SLS and quantity sufficient glycerin in water by continuous stirring for a period of 2 hr. NBT was dissolved in ethanol and the solution was added gently to carbapol 940, sodium lauryl sulphate, glycerin dispersion under continuous stirring. The dispersion was then allowed to hydrate and swell, adjusted the pH. The mixture was stirred gently until homogeneous gel was formed. All the samples were allowed to equilibrate for at least 24 hrs at room temperature.

Evaluation of topical gel

A) Physical Evaluation

Prepared and optimized batches of topical gel were evaluated by sensory evaluation for clarity, colour, homogeneity, presence of particles and fibres.

a) Spreadability

Spreadability was determined by an apparatus introduced by Mutimer et al. The apparatus consists of a wooden block provided by a pulley at one end and glass plate was fixed on the block. An excess of gel (about 2 gm) under study was placed on the lower plate. The upper plate was then subjected to a pull and time noted (in sec) required by the upper plate to cover a distance of 10 cm. A shorter the time interval better the spreadability.

b) Measurement of pH

The pH of the optimized gel formulation was determined with a digital pH meter at room temperature. The pH meter was calibrated by using standard buffer solution pH 4, 7 and 9.2. The gel (2.5 gm) was dispersed in 25

ml of purified water. Then pH meter was dipped in the gel solution and the pH was recorded.

c) Viscosity

The viscosities of optimized batches were determined using Brookfield's viscometer. The gel was placed in the sample holder and the suitable spindle (No.7) was lowered perpendicularly into the sample. The spindle was allowed to rotate at a constant optimum speed. The viscosity of the formulation was recorded at room temp.

d) Extrudability:

Extrudability was based upon the % quantity of gel extruded from tube on application of certain load. The formulation under study was filled in a clean, aluminum collapsible one-ounce tube with a nasal tip. It was then placed in between two glass slides and was clamped. When constant load was placed on slides, gel got extruded through the tip and extrudability was determined by weighing the gels.

B) % Drug content

Accurately weighed 0.5 gm of gel (equivalent to 10 mg of NBT) was transferred in 100 ml of volumetric flask, diluted with ethanol and sonicated. Then this solution was suitably diluted and absorbance was measured using UV against blank at 228 nm and drug content was determined.

C) Drug Release Study

a) In-vitro diffusion study: Cellophane membrane

A modified Franz diffusion cell was used for permeation studies. Cellophane membrane (no. 10, pore size 2.4 nm) soaked in phosphate buffer pH 6.8 for 24 hours before use. Cellophane membrane was placed in between donor and receptor compartment. Accurately weighed 1gm of gel was transferred to donor compartment and 25 ml of phosphate buffer pH 6.8 was filled in receptor compartment. The cell was agitated by a magnetic stirrer at 50 rpm at 37±1°C. Aliquots were withdrawn at specific interval of time and replaced with equal volume of fresh phosphate buffer pH 6.8. The samples were diluted suitably and absorbance was measured at 228 nm.

b) Ex-vivo diffusion study: Goat skin

Tissue Preparation

The skin of male goat, free from any visible disease was obtained immediately after sacrifice from a local slaughter house. It is transported to the laboratory in isotonic phosphate buffer (pH 6.8) and opened longitudinally and rinsed with same. Dorsal hairs and adhering subcutaneous fat was removed carefully. The excised skin was immersed in isotonic saline at 600C for 1min and ready for diffusion study. Phosphate buffer solution with pH 6.8 and maintained at 37 ± 0.50C kept in receptor compartment. 1 gm gel was placed in donor compartment, spreaded evenly and the permeation study was similarly carried out as that with cellophane membrane.

RESULTS & DISCUSSION

Drug Characterization

Melting point of NBT was found in the range of 800C-820C.

UV Spectroscopic Study

The maximum absorbance of NBT found at 228nm. So, λ max of NBT selected at 228nm.

Stability in Solvents

When NBT was analyzed in water, 0.1 N HCl and phosphate buffer with pH 6.8, no major changes observed.

Infra Red Spectroscopy

In the FTIR spectra analysis the characteristic peaks of NBT were observed at wave numbers 3062.25 cm-1, 2956.83 cm-1, 2848.37 cm-1, 2812.19 cm-1, 1705.04 cm-1, 1634.02 cm-1, 1485.64 cm-1, 1387, 1363.54, 1208.25, 957 cm-1, 895 cm-1 and 845 cm-1 for specific structural groups, confirming the purity of drug.

Phase Solubility Study

This study showed that the curve (Figure 2) obtained are AL type because of linear increase in solubility as the value of R2 closed to 1. The results of saturation solubility study indicated maximum increase in solubility with 1:3 with solvent wetting method.

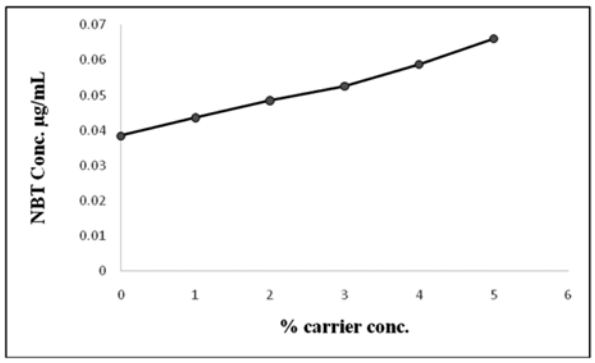

Figure No. 1: Phase Solubility study in aqueous solutions: NBT with urea

Percent Practical Yield & Drug Content

The practical yield and drug content for solvent wetting method was found as 91.59 ± 1.56 and 86.04 ± 1.08 %, respectively.

In Vitro Release Study

The in-vitro release of SDs showed significant increase in drug release, in comparison with pure crystalline NBT in dissolution medium. The maximum of dissolution enhancement was found with solvent wetting method.

FTIR

FTIR spectra showed some additional peaks in IR spectra which may be due to the carriers. While all other characteristic peaks of NBT are at the same wave number, indicated that there were no interactions of drug with carrier.

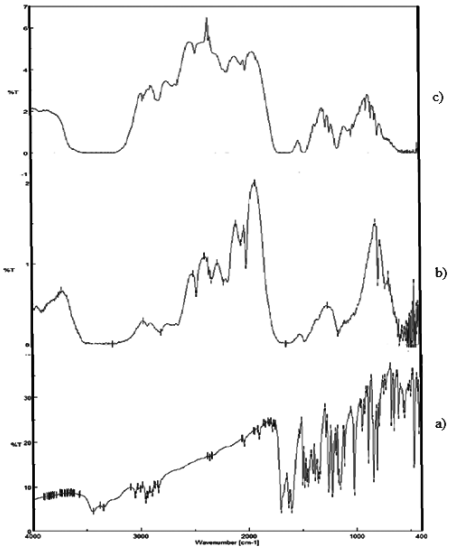

Figure No. 2: FTIR spectra a) NBT, b) Urea and c) NBT:Urea (1:3) SW

PXRD

PXRD pattern showed crystalline nature which was indicated by the numerous distinctive peaks at 2θ values are 19.26 and 26.50 in PXRD study of NBT. There were no numerous distinctive peaks seen in XRD pattern of SD. This showed that the high amount of NBT was dissolved in carrier in amorphous nature.

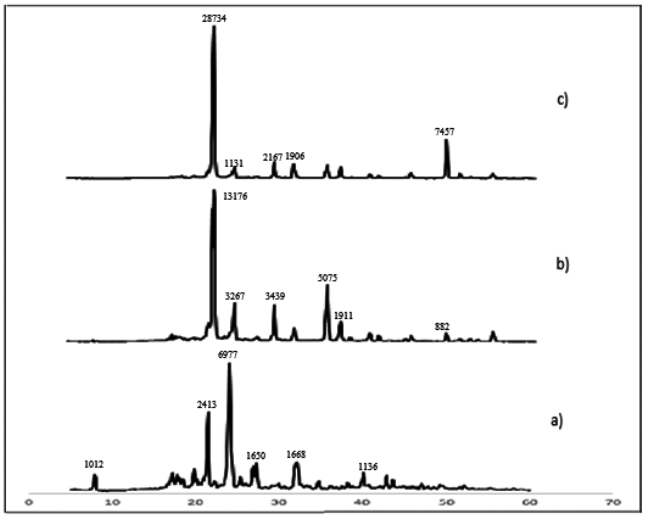

Figure No. 3: PXRD pattern for NBT: Urea ratio 1:3 - a) NBT, b) SW and c) PM

DSC

The DSC curve of NBT exhibited a sharp endothermic peak at 83.73°C due to fusion. Analogously, the thermal curve showed a single endothermic effect with a peak near about at 57.00 ºC, corresponding to its melting point. The DSC graph of SDs showed position of endothermic peak is shifted at 78.11 ºC (Ratio 1:1) and 75.17 ºC (Ratio 1:2) and 60.60 ºC (Ratio 1:3) respectively with decreased intensity than pure NBT. It indicates that crystalline nature of NBT gets transformed into amorphous carriers and thus melting of NBT became faster. The thermograms showed in figure 5.

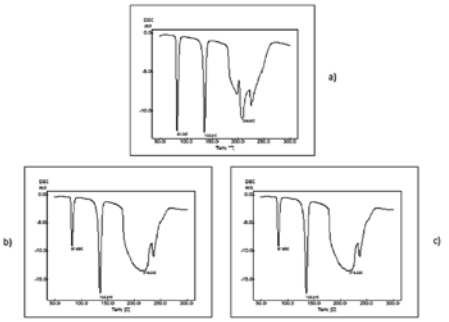

Figure No. 4: DSC thermograms of SD of NBT with urea in ratios a) 1:1, b) 1:2 and c) 1:3

Dissolution Study

Dissolution study of performed by using different ratios which were finalized after preliminary saturation solubility study. The release for cumulative % drug release obtained for all the formulations and pure NBT are tabulated. Figure 5 shows graphical presentation of drug release study. The dissolution rate of pure NBT was very poor and after 2 hrs i.e. 10.21 ± 1.39 %. While the dissolution rate of SDs after 2 hrs for SD of NBT: Urea (SW) with ratio 1:3 was found to be 35.49 ± 0.41.

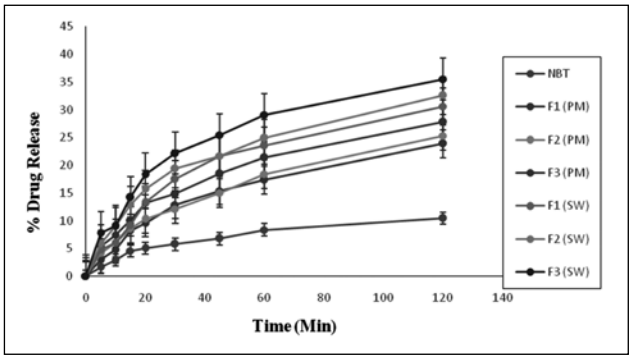

Figure No. 5: % Drug release profile of all ratios in distilled water NBT PM and SW with urea

Stability Study of SD

The stability study was carried out for SD prepared by PM and SW methods and parameters for study were drug content, in vitro release studies and FTIR. There was no degradation observed in stability studies. Thus, prepared SDs can be stored for one year.

Evaluation of Topical Gel

From the study of dissolution profile of all SDs the F6 batches were selected for further study as drug release of SW method with drug to carrier ratio 1:3 showed good release compared to another method with other ratios.

Physical Evaluation

The prepared gel formulations were examined visually for color and appearance. All formulations were clear. All batches of gel formulations showed good homogeneity with absence of lumps.

Spreadability

The value of Spreadability was found as 27.61 ±1.24 g.cm/sec for formulation F6.

Measurement of pH

The pH of formulations was in range of 6.91 ± 0.64 to 7.2± 0.36 which lies in the normal pH range of skin.

Viscosity

Viscosity of gel measured at 2, 4, 10 and 20 rpm and summarized in table no.5. Viscosities were proportional to the concentration of gelling agent. As rotating speed increased, the viscosity decreased which indicated shear thinning property.

Extrudability

Extrudability of all the formulations is higher than 80% and found 88.59% for formulation F6.

Drug content

The drug content of F6 formulation was in the range of 88.90 ± 1.77 % to 94.68 ± 2.31 %.

In-vitro drug diffusion study
a) Cellophane membrane

SDs which showed maximum drug release in distilled water was selected for further study of gel. Formulation batch F6 showed good release profile due to the lower HPMC K4M and higher Carbopol 940 content. Figure 6 shows graphical representation of release.

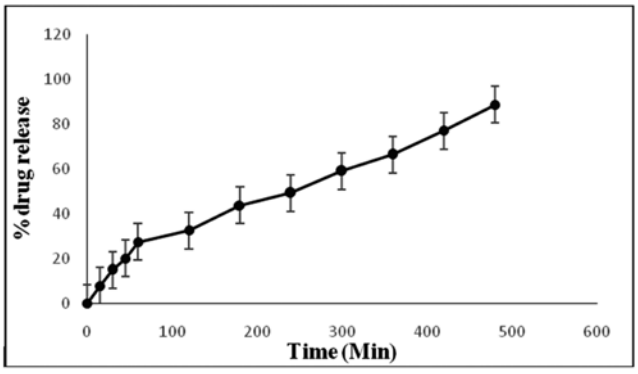

Figure No. 6: Diffusion study (Cellophane membrane) NBT + urea (ratio 1:3) F6 Batch

b) Ex-vivo drug permeation study:

Ex-vivo study was done only with best optimized formulations i.e. F6 and found that ex-vivo release was less than in-vitro release through both membranes. This decrease in drug release may be due to the fat content and thickness of Goat skin. Figure 7 show graphical presentation of release profile.

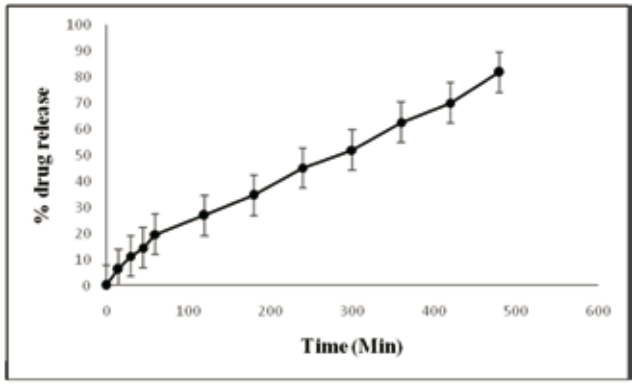

Figure No. 7: Diffusion Study (Goat skin) NBT + Urea (Ratio 1:3) F6 Batch

CONCLUSION

The solid dispersion obtained by solvent wetting method provides better control of drug release rate than physical mixture for same drug to polymer ratio. The water-soluble carrier urea and different techniques were studied to formulate solid dispersion, which gives improvement in solubility and drug release profile of nabumetone. The DSC, XRD and FTIR study had shown no interaction between nabumetone and carrier urea. The dispersion system is more efficient for preparation of nabumetone sustained-release mucoadhesive buccal patches which can reduce first pass metabolism of the drug with improved dissolution.

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Remedial Effect of Banana Peel Extract And Its Formulation

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ABSTRACT

Bananas belonging to the family Musaceae are one of the most important tropical fruits in the world market. Significant quantities of banana peels equivalent to 40% of the total weight of fresh banana are generated as a waste product in industries producing banana based products. Studies show that banana peels are good sources of polyphenols, carotenoids and other bioactive compounds which possess various beneficial effects on human health. However, there is limited information about the phytochemical and pharmacological properties of banana peel. Peels have been reported to possess antifungal and antibiotic components and used for the treatment of Acne. With these considerations the present work has been aimed to exploit the potential value of the peels of 'Poovan' variety of banana including phytochemical, antimicrobial and evaluation of Anti Acne activity by formulating as gel. It was observed that the peels of Poovan banana extract possessed significant antimicrobial activities. Also the gel prepared by using banana peel exhibited characteristic anti acne property when tested against *Propioni bacterium*. Hence from the present study it may be concluded that the banana peels can be tried in herbal formulations in future to provide an effective treatment against acne inducing bacteria and other microorganisms.

Key words: Banana peel, Antimicrobial activity, Anti acne property

INTRODUCTION

Bananas belonging to the family *Musaceae* are one of the most important tropical fruits in the world market. Banana peel is the outer covering of the banana fruit. Once the peel is removed, the fruit can be eaten raw or cooked and the peel is generally discarded. Peels form about 18-33% of the whole fruit and are reported to possess antifungal and antibiotic components and used for the treatment of Acne. Banana peels are the major by-products obtained during the processing of fruits and some studies show that these are good sources of polyphenols, carotenoids and other bioactive compounds which possess various beneficial effects on human health.1 Banana peel is rich in dietary fibre, proteins, essential amino acids, polyunsaturated fatty acids and potassium.2 However, there is limited information about the phytochemical and pharmacological properties of banana peel. Hence in this study, the Poovan variety of banana grown in Southern India namely *'Musa acuminata × Musa balbisiana'* determined with the aim of exploiting the potential value of the peels and its possible utilization including Phytochemical, Antimicrobial and Evaluation of Anti Acne activity by formulating as gel.

EXPERIMENTAL MATERIALS AND METHODS

Poovan Species:

Poovan banana is crossed species of Musa acuminata and Musa balbisiana (Family: Musaceae).Tamilnadu is the leading producer of Poovan cultivar commercially cultivated for leaf industry throughout Tamilnadu and in certain parts of Kerala.[3]

Preliminary Phytochemical Investigation of Peels of Poovan Banana Group:

Bananas were purchased from a local market in one lot and processed. Peels from banana namely Poovan (Musa acuminata× Musa balbisiana) were selected. Peels were washed with distilled water and dried in oven at 50 ± 1°C and powdered using a lab grinder and stored in air-tight jars till use.

Extraction of Plant Material:

About 100 g air dried powder of banana peels defatted with petroleum ether and extracted with ethanol using Soxhlet apparatus (Extractive value of 13.64 % w/w).

Phytochemical Examination:

The extracts were subjected to qualitative tests for the identification of the phytoconstituents present in it viz., alkaloids, saponins, glycosides, flavonoids, steroids[4,5].

Anti Microbial Studies:

Filter paper disc agar diffusion method was used for the in vitro evaluation of anti-microbial activity. The sterilized Whatmann filter paper No. 2 discs (6 mm diameter) were thoroughly moistened with the extract and the standard discs of Ciprofloxacin 5µg / disc and Nystatin 100 units /disc were used as standards for bacterial and fungal strains respectively. The inhibitory zone was measured with the help of antibiotic zone reader.[6]

Development of Herbal Gel:

Banana peel extract and Carbapol 934 were dissolved in sufficient quantity of water and kept overnight. To this sodium hydroxide was added to form a gel. Banana peel extract and Carbapol 934 gel were mixed together with vigorous stirring and kept in a beaker. The beaker was kept on a water bath and the temperature was allowed to reach above 500c. At the same time in another beaker weighed quantities of methyl paraben and propyl paraben were added in water and heated to dissolve. In another beaker weighed quantities of propylene glycol and polyethylene glycol were taken. Thus the mixtures obtained were finally mixed to obtain a gel. Then remaining quantity of purified water was added and pH was adjusted to 6.8 with 10% sodium hydroxide solution.7

Screening of Anti Acne Activity of Developed Gel:

Acne vulgaris is an inflammatory disease of sebaceous follicles of skin marked by comedones, papules and pustules and presence of bacteria Propionibacterium acne. The Anti acne activity for the developed gel was tested by disc diffusion method.8

RESULTS AND DISCUSSION

The alcoholic extract of banana peel was found to contain tannins, flavanoids and glycosides. From Table 1 it was observed that the standard drug Ciprofloxacin exhibited maximum zone of inhibition (28mm) against Staphylococcus aureus. Similarly test extract also exhibited maximum zone of inhibition 26mm against E.coli.

Table 1 Anti Microbial Activity of Extracts of Banana Peels

S.No	Name of the Organism	Zone of Inhibition (mm)			
		Alcohol Extract (µl)			Stan-dard
		100	200	500	
1	Staphylococcus aureus (NCIM 2079)	14	22	26	28
2	Bacillus subtilis	6	9	18	29
3	E.coli (NCIM 2065)	8	12	14	18
4	Klebsiella sp. (NCIM 2098)	12	15	17	28
5	Aspergillusniger (NCIM 105)	12	15	20	22
6	Candida albicans	10	15	16	20

Dilution : 100mcg, 200mcg, 500mcg.
Standard : Nystatin (Fungi)
Ciprofloxacin (Bacteria)

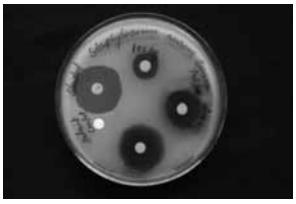

Fig.1 Resistanc against Staphylococcus aureus

Fig. 2 Resistance against Bacillus subtilis

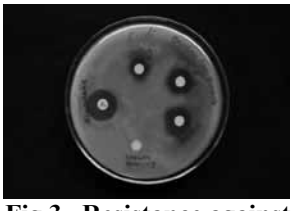

Fig.3 . Resistance against E.Coli

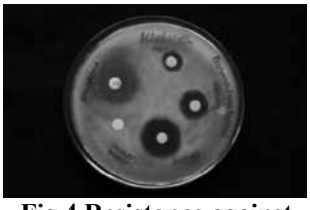

Fig.4 Resistance against Klebsiella sp.

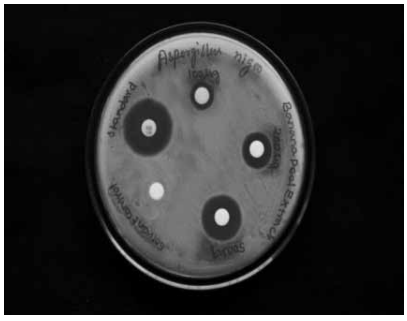

Fig.5 . Resistance against Aspergillus niger

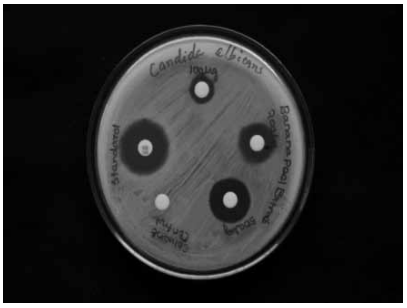

Fig.6 . Resistance against Candida albicans

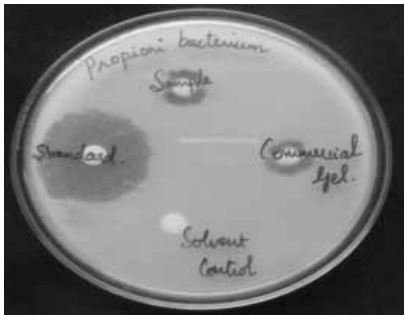

Fig.7.Anti microbial activity against Propioni bacterium acne

The zone of inhibition was found to be (29 mm) for standard drug against Bacillus subtilis and it was(18mm) for test extract. For Klebsiella sp. zone of inhibition was found to be (28mm) for standard drug and (17mm) against test extract Fig.1- 6. Also the results of this investigation showed that the formulation of banana peel extract had inhibitory effect on the P.acne (Fig.7) and was comparable to that of marketed preparation.

CONCLUSION

Banana peels are generally discarded and there is a significant amount of organic waste being generated. With a view to exploit banana peel as a source of valuable components, preliminary work has been carried out in the present study to investigate Banana Peels of Poovan variety. It was observed that the Peels of Poovan Banana group extracts possessed significant antimicrobial activities. Also the gel prepared by using banana peel exhibited characteristic anti acne property when tested against *Propionibacterium*. Hence the banana peels can be tried in herbal formulations in future to provide an effective treatment against acne inducing bacteria and other microorganisms thereby exhibiting anti-bacterial, anti-fungal properties and may provide various Healthfulness effects.

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Nine - Piperazinyl Acridine Derivatives - A Novel Class of Antibacterial Agents

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ABSTRACT

A novel series of 9-piperazinyl acridine derivatives were synthesised and characterised by IR, 1H NMR. 9-chloroacridine, 9-piperazin-1-yl acridine and their substituted compounds with varied substitutions were evaluated for in vitro antibacterial activity by measuring the minimum inhibitory concentration, all the derivatives exhibited significant to moderate antibacterial activity. This introductory research on 9-piperazinyl acridine derivatives, paves way for advanced mechanism antibacterial studies.

Key words: N-Phenylanthranilic acid, 9-piperazinyl acridine, antibacterial activity, benzyl derivatives, minimum inhibitory concentration

INTRODUCTION

Momentous attempt continues to be dedicated to the research, development and trade of novel antibacterial agents. Increasing problem of drug resistance by the existing class of pharmaceutical has imposed an urge in the exploring of novel chemical classes as anti-bacterial agents. Though novel target based inhibitory chemical classes have emerged, they failed in the clinical trials, due to lack of knowledge in the structural activity relationship of the drug[1]. Synthesis of acridine and their derivatives has attracted substantial attention from organic and medicinal analysts for countless years, since most of the natural sources have been proclaimed to acquire this heterocyclic nucleus[2]. Acridine is an alkaloid from anthracene. Commonly, known by the names of dibenzopyridine, 2,3,5,6-dibenzopyridine and 10 azaanthracene. Salts of acridine exhibits blue fluorescence[3].

In the present arena, enormous increase in drug resistance for bacterial infections has attracted the consideration towards acridine and its derivatives for novel therapeutic leads. During the last 20–30 years a large number of derivatives belonging to the general class of aniline acridine have been prepared and evaluated extensively as anti-malarial[4,5], anti-infammatory [6], antimicrobial [7] and anticancer [8,9,10] and antibacterial agents [11,12].

Inspired by the wide range of versatile chemotherapeutic activities of acridine, a significant amount of research activity has been directed toward this class in recent years. Hence the present work describes methods for the synthesis of novel substituted acridine derivatives as antibacterial agents. Characterization of the synthesized compounds was carried out using Infrared spectroscopy and NMR interpretations. Anti-bacterial potential has been studied based on the minimum inhibitory concentration in accordance with the standard drug.

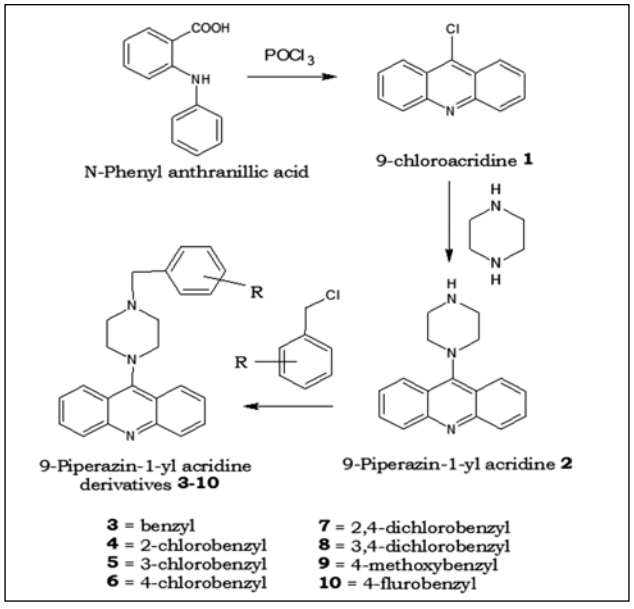
2. Experiment

All chemicals used were of reagent grade and purified as per need of the reaction. Progress of the reaction was monitored by TLC using hexane:ethylacetate (7:3)

as mobile phase.

2.1. Chemistry

9-Choloro acridine1 derivatives synthesized by cyclization of N-phenyl anthranillic acid with freshly distilled phosphorus oxychloride under nitrogen atmosphere (Scheme 1). Further, it was treated with piperazine to obtain 9-piperazi-1-yl acridine2. The compounds are reacted with several un-substituted benzyl chloride to yield derivatives 3-10.



Scheme. 1 Synthesis of 9-piperzinyl acridine derivatives **3-10**

2.2. Mechanism of reactions

The mechanism involves in the first step is cyclisation reaction. The second and final step is simple condensation and completes with liberation of hydrochloric acid. The final step involves with various benzyl chlorides to yield final products.

2.2.1. Synthesis of 9-Chloroacridine 1

N-Phenyl anthranillic acid (213 mg, 1mmol) was suspended in phosphorous oxychloride (0.5 ml, 5 mmol) and heated at 100C for 10 mins. The mixture was poured onto ice and neutralised dropwise to pH 7 at 0C with cold 2M NaOH (6.5 ml). The white precipitate obtained was filtered, dried by suction and sublimed at 60C. Caution: The Procedure should be carried out in an efficient hood and exposure to POCl3 should be avoided.

2.2.2. Synthesis of 9-piperazin-1-yl acridine 2

In DMF - 1 mmol of 9-Chloro acridine was suspended and was mixed by shaking, then 1.5 mmol K2CO3 and 1.2 mmol piperazine were added to the solution and kept for magnetic stirring for 10 hours at room temperature followed by refrigeration. The mixture was poured onto ice water and stirred well. The solution was filtered for the solid settles and then it was dried in an oven to get a fine powder. The product was recrystallized with ethanol.

2.2.3. General method of synthesis of 3-10

To a solution of 9-(piperazin-1-yl)acridine (263mg,1mmol) in 10ml of DCM taken in a round bottomed flask, pyridine-0.1ml and various (un) substituted benzyl chloride 0.1ml (1 mmol) were added and the reaction mixture was stirred for 1 hr at room temperature. Then the mixture was extracted with 10 ml of 1% CuSO4 solution subsequently with ice cold water. The organic layer was separated and filtered through NaSO4 and evaporated to get the product.

2.3.1. Synthesis of 9-chloroacridine1,

Green colored product, yield – 85%, Rf – 0.32, m.p. 117 °C (lit.117–118 °C).IR (KBr, cm-1) 3043, 1628, 1590, 1447, 1404, 1261, 1080, 1019, 804, 757.1H-NMR d (d6-DMSO, ppm) 8.43 (d, 1H, J¼8.7 Hz), 8.22 (d, 1H, J¼8.7 Hz), 7.81 (dd, 1H, J¼7.3 Hz), 7.63 (dd, 1H, J¼7.3 Hz).

2.3.2.Synthesis of 9-(piperazin-1-yl)acridine2,

Yellowish green colored product, yield – 74%, Rf – 0.72, m.p. 129 °C. IR (KBr, cm-1) 3028, 1637, 1571, 1455, 1399, 1252, 1085, 1022, 809, 762.1H-NMR d (d6-DMSO,ppm) 8.40 (d, 2H aromatic), 8.24 (d, 2H aromatic), 7.83 (t, 2H, aromatic), 7.65 (m, 2H, aromatic).

2.3.3. Synthesis of 9-(4-benzylpiperazin-1-yl) acridine3,

White amorphous solid, yield – 62%, Rf – 0.18, m.p. 122 °C. IR (KBr, cm-1) 3030, 1639, 1579, 1463, 1406, 1262, 1091, 1028, 812, 752.1H-NMR d (d6-DMSO, ppm) 8.24–8.22 (d, 4H, aromatic), 7.64–7.62 (d, 1H, aromatic), 7.55–7.49 (q, 3H, aromatic), 7.39–7.34 (t, 2H, aromatic), 7.13–7.09 (t, 2H, aromatic), 2.42 (s, 2H, methylene)

2.3.4. Synthesis of 9-[4-(2-chlorobenzyl)piperazin-1-yl]acridine 4,

Green coloured crystalline solid, yield – 37%, Rf – 0.27, m.p. 132 °C. IR (KBr, cm-1) 3042, 1640, 1562, 1471, 1401, 1261, 1085, 1020, 801, 761.1H-NMR d (d6-DMSO, ppm) 8.20–8.18 (d, 4H, aromatic), 7.60–7.58 (d, 1H, aromatic), 7.37–7.32 (t, 2H, aromatic), 7.11–7.08 (t, 2H, aromatic), 2.40 (s, 2H, methylene)

2.3.5. Synthesis of 9-[4-(3-chlorobenzyl)piperazin-1-yl]acridine 5,

Algae green colored crystalline solid, yield – 27%, Rf – 0.29, m.p. 116 °C. IR (KBr, cm-1) 3032, 1649, 1571, 1480, 1399, 1255, 1086, 1022, 806, 767.1H-NMR d (d6-DMSO, ppm) 8.22–8.20 (d, 4H, aromatic), 7.62 (d, 1H, aromatic), 7.53–7.47 (q, 3H, aromatic), 7.15–7.11 (t, 2H, aromatic), 2.45 (s, 2H, methylene)

2.3.6.Synthesis of 9-[4-(4-chlorobenzyl)piperazin-1-yl]acridine 6,

Dark green colored crystalline solid, yield – 31%, Rf – 0.35, m.p. 124 °C. IR (KBr, cm-1) 3318, 3023, 2881, 2740, 1638, 1581, 1163, 1114, 748, 698, 655. 1H-NMR d (d6-DMSO, ppm) 8.18–8.16 (d, 4H, aromatic), 7.62 (d, 1H, aromatic), 7.37–7.32 (t, 2H, aromatic), 7.09–7.05 (t, 2H, aromatic), 2.38 (s, 2H, methylene)

2.3.7.Synthesis of 9-[4-(2,4-chlorobenzyl)piperazin-1-yl]acridine 7,

Dark green colored crystalline solid, yield – 31%, Rf – 0.35, m.p. 124 °C. IR (KBr, cm-1) 3428, 2899, 2748, 1629, 1581, 1547, 1513, 1442, 1231, 1159, 1091, 1019, 756, 683. 1H-NMR d (d6-DMSO, ppm)8.22–8.20 (d, 4H, aromatic), 7.62–7.60 (d, 1H, aromatic), 7.53–7.47 (q, 3H, aromatic), 7.41–7.36 (t, 2H, aromatic), 7.15–7.11 (t, 2H, aromatic), 2.45 (s, 2H, methylene)

2.3.8.Synthesis of 9-[4-(3,4-chlorobenzyl)piperazin-1-yl]acridine 8,

Dark green colored crystalline solid, yield – 31%, Rf – 0.35, m.p. 124 °C. IR (KBr, cm-1) 3318, 3023, 2881, 2740, 1638, 1581, 1163, 1114, 748, 698, 655, 655. 1H-NMR d (d6-DMSO, ppm) 8.17–8.19 (d, 4H, aromatic), 7.54–7.52 (d, 1H, aromatic), 7.45–7.42 (q, 3H, aromatic), 7.33–7.30 (t, 2H, aromatic), 7.08–7.04 (t, 2H, aromatic), 2.36 (s, 2H, methylene)

2.3.9.Synthesis of 9-[4-(4-methoxybenzyl)piperazin-1-yl]acridine9,

Dark green colored crystalline solid, yield – 31%, Rf – 0.35, m.p. 124 °C. IR (KBr, cm-1) 3378, 3122, 1612, 1582, 1566, 1518, 1442, 1364, 1262, 1124, 1082, 988, 974. 1H-NMR d (d6-DMSO, ppm) 8.28–8.24 (d, 4H, aromatic), 7.69–7.67 (d, 1H, aromatic), 7.61–7.57 (q, 3H, aromatic), 7.44–7.48 (t, 2H, aromatic), 7.18–7.14 (t, 2H, aromatic), 2.55 (s, 2H, methylene)

2.3.10.Synthesis of 9-[4-(4-flurobenzyl)piperazin-1-yl]acridine 10,

Dark green colored crystalline solid, yield – 31%, Rf – 0.35, m.p. 124 °C. IR (KBr, cm-1) 3398, 3017, 2924, 2834, 1598, 1573, 1536, 1431, 1308, 1228, 1189, 1012, 985, 946. 1H-NMR d (d6-DMSO, ppm) 8.23–8.21 (d, 4H, aromatic), 7.63–7.60 (d, 1H, aromatic), 7.54–7.48 (q, 3H, aromatic), 7.40–7.35 (t, 2H, aromatic), 7.12–7.08 (t, 2H, aromatic), 2.44 (s, 2H, methylene)

3. Antibacterial activity

All the synthesised compounds were screened in vitro for their antibacterial activity against Staphylococcus aureus, Escherichia coli, Bacillus pumillus, Salmonella typhi, Klebsiella pneumonia, Pseudomonas aeruginosa by agar dilution method at 100µg/mL concentration using DMSO as a control. After 24 h of incubation at 37º C the MIC was measured. The results are tabulated in Table 1.

Table-1 Antibacterial activity of 9-piperzin-1-yl acridine derivatives

Compd. No	MIC in µg/mL					
	S. aureus	E. coli	B. pumillus	S. typhi	K. pneumonia	P. aeruginosa
3	12.5	50	50	25	25	50
4	50	25	50	100	25	12.5
5	25	12.5	50	50	50	25
6	25	6.25	50	50	12.5	12.5
7	50	12.5	12.5	50	25	25
8	100	25	50	100	50	50
9	50	12.5	12.5	50	25	25
10	25	25	50	50	50	25

4. Results and Discussion

The moiety employed for the synthesis of 9-(piperazin-1-yl)acridine derivatives, where adjacent to reactive chlorine possess –CH2 functional group facilitates the chlorine atom as the better leaving group. TLC and spectral data confirmed the formation of product and the yields were found to be good. The products formation under same reaction conditions shows that chlorine substituted on aromatic system of the derivative gave fewer yields compared with other derivatives.

This may be due to the release of electron from chlorine followed by delocalization of pi electrons and further make the aromatic system electron deficient due to its (chlorine atom) high electro-negativity. This effect hinders the reactive methylene group to a significant level, for the formation of product. Also, mono chloro substituted derivative was high when compared with dichloro substitution. In case of 4-methoxy 9and 4-fluro 10derivative, the yield is high may be due to the electron donating nature of 4th position group -OCH3 and –F group which facilitates the leaving group as a better nucleophile. The synthesized compounds were tested for antibacterial activity, in which 4-chloro derivative 6showed best antibacterial activity against E. coli compared with other derivatives.

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A Comparative Study on Paracetamol Versus Ibuprofen In Reducing The Temperature Among The Children

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ABSTRACT

Objective: To compare the efficacy and safety of paracetamol versus ibuprofen monotherapy in reducing the temperature among the children in the age group between 6 months to 15 years.

Materials and methods: 100 children were enrolled in the study and divided into two groups for a period of 2 years. One group of children were treated with only Paracetamol of 15mg/kg/dose every 6 hours and another group of children were treated with Ibuprofen of 10mg/kg/dose every 8 hours. Study subjects were observed in wards from the time of admission for at least 24 hours or till clinical cure was achieved. Independent 't' test was used for statistical analysis and $P<0.05$ was considered as statistically significant. The results were expressed as mean ± SE.

Results: It was observed that no significant difference in the temperature between the groups ($P>0.05$). While comparing the time taken to reach baseline temperature, children who were treated with Ibuprofen was 60 minutes, which reveals the significant effect ($P<0.05$) than acetaminophen group. The total number of doses needed to recover completely from febrile phase and mean duration of hospital stay was significantly ($P<0.05$) lower in ibuprofen group. It was found that significant reduction in temperature ($P<0.05$) before and after treatment. There was incidence of abdominal pain in ibuprofen group whereas no adverse event in paracetamol group.

Conclusion: Although both acetaminophen and Ibuprofen proved to be effective antipyretic agents and were found to be well tolerated by study subjects, Ibuprofen has better antipyretic activity than acetaminophen.

Key words: Fever, Acetaminophen, Ibuprofen, Monotherapy, baseline temperature.

INTRODUCTION

Fever is one of the most common presenting signs of illness in office based primary care paediatric practice, accounting for 19% to 30% of visit. Fever is an elevation of body temperature above the normal circadian range as a result of a change in thermoregulatory centre located in hypothalamus. Fever may be defined as an AM temperature greater than 37.20°C (98.90 F) or a PM temperature of greater than 37.70°C (99.90 F). The rectal temperature is 0.60°C (10 F) higher than the oral temperature. The oral temperature is same as freshly voided urine. Hyperthermia (above 41.60°C or >107.0 F) is an elevation of core temperature without the elevation of the hypothalamic set point[1]. From the time of Hippocrates to 19th century, fever was perceived beneficial until when Claude Bernard showed it fatal in animals when body temperature raised 5-60°C in animals. Most of the drugs used as antipyretics reduce the levels of prostaglandin synthesis in brain and thus, setting the thermostat back to normal set point[2]. The pharmacological therapy includes different classes of drugs with analgesic – antipyretic effects only. Acetaminophen and ibuprofen are the only two antipyretic agents recommended for use in paediatrics, but combining or alternating use of these agents is not recommended. It was reported that an alternating treatment regimen of acetaminophen (12.5 mg/kg per dose) and ibuprofen (5 mg/kg per dose) every 4 hours for 3 days, regardless of the initial loading medication,

it is more effective than monotherapy in lowering fever in infants and children[3].

Paracetamol:

Paracetamol (acetaminophen) is a popular 'home' medicine in widely use and enjoys the clinical standing of over 50 years. It's analgesic effect is equal to that of aspirin but in clinical doses has no anti-inflammatory effect[4]. Paracetamol also has potential side effects and may cause severe hypersensitivity reactions. It has not been demonstrated by controlled trials to benefit children with ARI. There are reports to suggest about slow clearance of plasmodium from blood. Committee on Infectious Diseases of American Academy of Paediatrics has recommended avoiding the use of any antipyretics in management of patients with enteric fever due to possibility of rapid effervescence and development of shock[5]. Besides, the neonates and infants handle paracetamol less efficiently and are capable of forming the reactive intermediate metabolite that causes hepatocellular damage, particularly after multiple dosing. They have an immature glucouronide conjugation system, but the rate constant for the sulphotion metabolic pathway is larger than in older children, and this is the most important route of metabolism[6]. Children are especially vulnerable to accidental exposure due to non-availability of childproof containers in India. The main clinical features of acute toxicity include anorexia, vomiting, abdominal pain, jaundice, hematuria and metabolic acidosis. Diagnosis is based on history and laboratory findings of acidosis and abnormal liver function tests[7]. N-acetylcysteine is used as specific antidote.

IBUPROFEN:

It is a propionic acid derivative widely use for its analgesic and anti-inflammatory action. It is well absorbed orally and achieves maximum plasma concentration in 1-2 hour. The t1/2 of ibuprofen is 2 hours. Ibuprofen binds extensively in a concentration-dependent manner to plasma albumin. In the doses greater than 600mg there is an increase in unbound fraction of the drug, leading to increased clearance of ibuprofen and a reduced AUC of the total drug[8]. Ibuprofen is eliminated by following biotransformation to glucouronide conjugate metabolites that are excreted in urine, with little of the drug being eliminated unchanged. The excretion of conjugates may be tied to renal function and the accumulation of conjugates occurs in end stage renal disease. Hepatic disease and cystic fibrosis can alter the disposition kinetics of ibuprofen. Substantial concentrations of ibuprofen are attained in synovial fluid, which is proposed site of action for non-steroidal anti-inflammatory drugs[9]. Ibuprofen is not excreted in significant concentrations into breast milk. Significant drug interactions have been demonstrated for aspirin (acetyl salicylic acid), cholestyramine and methotrexate. A relationship between ibuprofen plasma concentrations and analgesic and antipyretic effects have been elucidated. Ibuprofen has been used alone or in combination with paracetamol[10]. It received FDA approval in May 1984. Studies found that ibuprofen is beneficial over paracetamol in reducing fever in children. Both drugs have been found efficacious in reducing fever with minimum side effects. Ibuprofen in doses of 6mg/kg/dose and 7mg/kg/dose have efficacy equal to 10 and 15 mg/kg/dose of aspirin and 8-12 mg/kg/dose of paracetamol . Its efficacy in doses of 10 mg/kg/dose is equal to 12.5 mg/kg/dose of paracetamol and action is longer than paracetamol[11-12]. Acetaminophen and ibuprofen are widely prescribed in children and are the most frequently used over the counter analgesics and antipyretics and yet their relative efficacy and safety is uncertain, when used in recommended doses. Although studies have concluded ibuprofen to be the superior analgesic and antipyretic, literature review typically have concluded that the drugs were equally effective but that acetaminophen should be used because its safety seemed more assured. There is also a difference observed between subjective and objective findings in groups treated with Ibuprofen versus paracetamol in pediatric fever. Though no statistically significant differences were observed in the end points on examination and tolerability of both agents were similar, compared with parent of paracetamol group and significantly more parent of ibuprofen group rated the drug as very efficacious.

Institutions differ greatly as to which agent is used as a first line antipyretic. No clear guidelines exist regarding the choice of ibuprofen or acetaminophen in reducing fever in children. Also inappropriate dosing or overdosing is not uncommon. So it was decided to conduct a study to compare the efficacy and safety of acetaminophen

versus ibuprofen in reducing the temperature in febrile children. The aim of our study was to compare the efficacy and safety of acetaminophen versus ibuprofen monotherapy in reducing the temperature among the children in the age group between 6 months to 15 years.

Material and Methods:

A prospective randomized observational study was done in the department of Pediatrics of a private corporate hospital, Coimbatore, India and the study was approved by Institutional Ethics Committee on December 2014. KMCH Ethics Committee (Reg. No: ECR/ 112 / inst / TN / 2013.) All children within the age group of 6 months to 15 years who fulfilled the standard definition of fever (auxiliary temperature above 37.50° C or 99.0 F) irrespective of its etiology were included in the study and Independent 't' test was used for statistical analysis and 'P' value of <0.05 was considered statistically significant. The results were expressed as mean SD. SPSS software were used for analysis. For further study, populations were divided into two groups, Group's 'A' were treated with acetaminophen monotherapy and Group 'B' treated with ibuprofen monotherapy. One group of children treated with oral Paracetamol of 15mg/kg/dose every 6 hours and another group were treated with oral Ibuprofen of 10mg/kg/dose every 8 hours.

Exclusion criteria: Children with following criteria were not included in the study

- Age less than 6 months and more than 15 years
- Children, who were already on antibiotics like amoxicillin, co-amoxiclav, ceftriaxone, cefixime or having exposure to antibiotics within 10 days before presentation to hospital.
- Children with past history of known malignancies
- Children with following medical history of any of the following
  - Gastro intestinal bleeding
  - Known allergy to any of the antipyretic agents
  - Bronchial asthma
  - Bronchiolitis.

Results:

The initial temperatures measured in the two groups were not statistically significant. The time taken to reach baseline temperature was 60 minutes earlier in ibuprofen group and this is statistically significant. The total number of doses needed to recover completely from febrile phase and mean duration of hospital stay was significantly lower in ibuprofen group. The temperature level was lowered by ibuprofen group was also statistically significant. The study compared the efficacy and safety of paracetamol and ibuprofen monotherapy in treatment of fever irrespective of etiology among the children in the age group between 6 months to 15 years. The baseline temperature was measured on admission in both groups. It was observed that children who were randomly selected for acetaminophen group showed 102.6 ± 0.16 and 102.6 ± 0.16 for the children assigned for ibuprofen group. It reveals that there was no significant difference between the group (P>0.05) on admission. It was observed that 127.6 ± 0.06 minutes taken to reach baseline temperature among children treated with acetaminophen and 120 ± 0.21 minutes for ibuprofen. It reveals that ibuprofen has significant impact(P<0.01) on reduction in temperature after receiving first dose. The total number of doses needed to recover completely from febrile phase and mean duration of horizontal stay was significantly lower in children treated with ibuprofen. After four hours of drug administration, ibuprofen has an average of 4.23 ± 0.08 and acetaminophen with 3.75 ± 0.12 temperature, which shows ibuprofen has significant impact on reduction in temperature than acetaminophen (P<0.01). There was incidence of abdominal pain in ibuprofen group whereas no adverse event in acetaminophen group.

Table: 1 Comparison of Children treated with Acetaminophen and Ibuprofen

S.No	Descriptions	Mean ± SE		P Value
		Acetaminophen	Ibuprofen	
1	Temperature at the time of admission	102.6 ± 0.16	102.6 ± 0.16	0.97
2	Time taken to reach baseline temperature (After receiving 1st dose)	127.6 ± 0.06	120 ± 0.21	0.002*
3	Total number of doses required to reach baseline temperature	7.76 ± 0.32	5.4 ± 0.18	0.001*
4	Reduction in temperature after four hours of administration	3.75 ± 0.12	4.23 ± 0.08	0.002*

***P<0.05**

Discussion :

It was reported that two regimens were equally effective and equally tolerated in febrile children. Lower ibuprofen doses (2.5 and 5 mg/kg) were less effective than acetaminophen and 10-mg/kg ibuprofen therapy after the initial dose but at least equally effective as these two higher-dose regimens13. Our study included children of age group between 6 months to 15 years with a mean initial temperature of 102.5 F. Mean weight of entire study population was in the range between 15-18kgs. The results of our study showed significant difference between antipyretic effect of acetaminophen and ibuprofen. Previous studies reported that efficacy and effectiveness of acetaminophen and ibuprofen in their recommended dosages are similar, with slightly more beneficial effects shown with ibuprofen14-15. From our study, it was found that children receiving ibuprofen were found to reach baseline temperature following the first dose of the drug around 60 minutes earlier than children receiving acetaminophen. This shows that ibuprofen has lowered the temperature to the baseline faster than acetaminophen. Our study has few limitations like utilization of antibiotics were not concurrently studied.

Conclusion:

Both acetaminophen and ibuprofen were found to be effective antipyretic agents. It was observed that efficacy of ibuprofen was superior to acetaminophen in terms of taken to bring down the temperature to baseline and also requires reduced number of doses which proves its longer duration of action. Both drugs were found to be well tolerated by study subjective. The overall antipyretic effect of acetaminophen and ibuprofen were not found similar in our study with ibuprofen being more effective than acetaminophen. Thus, we conclude that ibuprofen appears to be the better antipyretic drug of choice in febrile children irrespective of etiology.

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