International Journal of Phytopharmacology
Research Article

www.onlineijp.com

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

A NOVEL HYDROGEL FORMULATION COMPRISING DEXAMETHASONE AND METRONIDAZOLE FOR THE EFFECTIVE TREATMENT OF ORAL MUCOSITIS IN CANCER PATIENTS

Junaid Abdul Majeed M.P.* and Jisha Mohanan

College of Pharmaceutical Science, Govt. Medical College, Kozhikode-673008, India.

ABSTRACT

Oral mucositis refers to erythematous and ulcerative lesions of the oral mucosa observed in patients with cancer being treated with chemotherapy and/or radiation therapy. Because of insufficient detection methods, cancer become one of the health problem, it is mainly caused by both internal and external factors like inherited mutations, hormonal imbalance, metabolic mutations, tobacco, radiation, chemicals etc[1].Various new drug designing techniques are used for diagnosis and cure. In recent years, the era of computer aided drug design has widely helpful to enhance our understanding of complex biological process and protein ligand interactions. Lesions of oral mucositis are often very painful and compromise nutrition and oral hygiene as well as increase risk for local and systemic infection. At the institute of pain and palliative Medicine, Medical College,Malabar area in Kerala. The patients who are suffering from oral mucositis are treated with an extemporaneous preparation for the past 7 years. It's a liquid mixture of dexamethasone and metronidazole tablets which are applied over the painful oral mucositis by grinding the tablets using mortar and pestle and then by mixing with glycerin and multivitamin syrup. But the present challenge is the absence of an appropriate formulation. When applied in the powder form patients are having difficulty in ingesting food. So this study is to convert an extemporaneous preparation 'gudallurmix' into a gel formulation,in order to improve the ease of administration, increase patient acceptability and to reduce the pain and discomforts of the patients. So, this formulation may prove to be a boon to a population who suffer a lot of pain in their lives.

Key words: Cancer, Oral mucositis, Dexamethasone, Metronidazole.

Corresponding Author: **Junaid Abdul Majeed M.P.** Email: junu.majeed@yahoo.com

INTRODUCTION

Oxidative stress is produced as a result of overproduction of free radicles or due to defecint antioxidant defence systems that can produce tissue injury[1]. The drug discovery, its development and its commercialization is a tedious and time consuming process. Only small amount of drugs which has been synthesized will be examined in clinics and among them few will be marketed[2].Oral mucositis refers to erythematous and ulcerative lesions of the oral mucosa observed in patients with cancer being treated with chemotherapy and/or radiation therapy. Lesions of oral mucositis are often very painful and compromise nutrition and oral hygiene as well as increase risk for local and systemic infection. Patients treated with radiation therapy for head and neck cancer typically receive an approximately 200 cGy daily dose of radiation, five days per week, for 5–7 continuous weeks. Almost all such patients develops some degree of oral mucositis. Oral mucositis can be very painful and can significantly affect nutritional intake,mouth care,and quality of life. For

Access this article online

DOI: http://onlineijp.com/

DOI: http://dx.doi.org/10.21276/ijp.2020.11.4.1

Quick Response code

Received:05.02.20	Revised:22.02.20	Accepted:15.03.20

patients receiving high-dose chemotherapy prior to hematopoietic cell transplantation, oral mucositis has been reported to be the single most debilitating complication of transplantation. Infections associated with the oralmucositis lesions can cause life-threatening systemic sepsis during periods of profound immunosuppresion. The majority of patients receiving radiation therapy for head and neck cancer are unable to continue eating by mouth due to mucositis pain and often receive nutrition through a gastrostomy tube or intravenous line.It has been demonstrated that patients with oral mucositis are significantly more likely to have severe pain and a weight loss of ≥5%[3,4.] Gel formulation is selected because it improves the ease of administration, increase patient acceptability and better bioavailability of the drugs may be expected there by it may lead to improved therapeutic outcomes. Dexamethasone is classified as a corticosteroid (more precisely a glucocorticosteroid), and has mainly used in the treatment of cancer. It works by decreasing inflammation (swelling) and is also used in the short-term treatment of nausea caused by chemotherapy. Dexamethasone is a steroid that prevents the release of substances in the body that cause inflammation and it is used to treat many different conditions such as allergic disorders, skin conditions, ulcerative colitis, arthritis, lupus, psoriasis, etc. Metronidazole (MTZ) is an itroimidazole antibiotic used mainly for the treatment of infections caused by susceptible organisms, particularly anaerobic bacteria and protozoa. Apart from its antibiotic effect it is also having amoebicidal and antiprotozoal effects[5].

MATERIALS AND METHODS

Materials

Dexamethaone and Metronidazole were purchased from Sigma Aldrich. Carbopol 940 was produced from Oxford Laboratory, Mumbai. Triethanolamine and Xanthan gum were from Astron chemicals, India. Glycerine was from Universal Chemicals and solvents used were of analytical grade.

Method

Required quantities of carbopol was taken and soaked in a small quantity of distilled water. After the complete wetting of the polymer, glycerine was added. The mixture was homogenised in a mortar and pestle. To the above mixture, xanthan gum and remaining quantity of distilled was added and heated to 80°C in a water bath. After bringing to the room temperature Dexamethasone and metronidazole was added and mixed in a homogeniser. After that, gelation was brought about by the addition of triethanolamine. During the addition of the triethanolamine the viscosity of the preparation was increased until a off white gel was obtained. The gel was then filled into metal tubes and was sealed.

Preformulation studies and compatibility studies of Dexamethasone and Metronidazole with the excipients

Characterization studies for the drugs, Dexamethazone and Metronidazole (IR spectroscopic analysis, the melting point by capillary melting method, Loss on drying and saturation solubility study) and the Drug excipient studies using Fourier transform infrared spectroscopy (FTIR) of drugs, Dexamethasone and Metronidazole with the excipients were performed prior to the preparation of the hydrogel. Differential scanning calorimetric analysis (DSC) were also performed in order to characterise the drug-excipient compatibility.

Evaluation of the formulated hydrogel

Determination of pH

The pH of gel was checked by using a digital pH meter at room temperature. Initially, the pH meter was calibrated using standard buffers. 10g of the gel was dispersed in a solvent and then pH meter was dipped in the dispersion and there by pH was noted

Determination of viscosity

The viscosity of the gels prepared was determined using Brookfield viscometer model (LVDV - II+), the gel sample was filled in the sample holder and the particular spindle immersed into the sample, the spindle is attached to the viscometer and then it is allowed to rotate at a particular speed then viscosity of the formulation was measured after 2 minutes.

Spreadability[6]

Spreadability of the formulations was determined by an apparatus suggested by **mutimer *et al., 1998*[6] .** It consists of a wooden block which was provided by a pulley at one end. A rectangular ground glass plate was fixed on the block. An excess of gels (about 2g) under study was placed on this ground plate. The gel was then sandwiched between this plate and another glass plate having the dimensions of the ground plate and provided with the hook. A 300g weight was placed on the top of two plates for five minutes to expel air and to provide a uniform film of the gel between the plates. Excess of the gel was scrapped off from the edges. The top plate was then subject to pull of 30g with the help of a string attached to the hook and time (in seconds) required by the top plate to cover a distance of 10cm be noted. A shorter the time interval indicates better spreadability

Grittiness[7]

All the formulations were evaluated microscopically for the presence of any appreciable particulate matter which was seen under light microscope.

Homogeneity [8,9]

After the gels have been set in the container, all developed gels were tested for homogeneity by visual inspection.

Extrudability[10]

The apparatus used for extrudability was suitably fabricated in laboratory .It consists of a wooden block inclined at an angle of 45°[10] fittedwith a thin, long metal strip (tin) at one end,while the other end was free. The Aluminium tube containing 12 grams of gel was positioned on inclined surface of wooden block. 1 Kg weight was placed on free end of the Aluminium strip and was just touched for 30 seconds. The quantity of gel extruded from each tube was noted.

Determination of,drug,content uniformity

Required quantity 1g of the formulation was taken 100ml volumetric flask and dissolved in 10ml of ethanol and the final volume was adjusted with phosphate buffer of pH 7.2 with vigorous shaking and the content was filtered through,a suitable filter paper. An aliquot1ml was pipette out from the filtrate and suitably dilute dinphosphate buffer to 10ml standard flask. The content of dexamethasone and metronidazole was determined by using u.v at 242 and 320nm respectively. The blank solution was also prepared in the same manner above using gel without drug.

HET – CAM Test[11,12]

The Hen's Egg Test on the Chorioallantoic membrane (HET – CAM) is an *in vitro* method to determine the irritation to the mucous membrane by using Chorioallantoic membrane of embryonated hen's egg. Its well-developed vascularization provides an ideal model for studies of potential irritation to mucous membrane caused by transdermal dosage forms like gels, ointments etc..This test assesses the damage to this membrane to determine the potential irritation to the mucous membrane

Treatment:

3 eggs per 3 groups are used in the study

Each groups contain – 1 negative control, 1 positive control and 1 test.

Fertilized hen's eggs (9 eggs)

↓

Kept for 9 days in BOD incubator at a temperature of 38°°C. Determine the air sac and then remove the inner membrane by using dentist rotating blade

↓

0.3 mL of Nacl solution is used as a negative control**,** are applied on the CAM**.**

0.1 N NaoH 0.3 mL are applied on the Chorioallantoic membrane as a positive control, a severe response is expected. 0.3 ml of formulation F4 is applied on to the CAM as a Test.

↓

Effect is assessed within 5 min.

End Point: Redness, Irritation ***In vitro*** **antibacterial Activity**[13,14]

Metronidazole is a nitroimidazole having antibacterial activity mostly against Gram -ve bacteria[56]. The antibacterial activity of gel formulation was performed on Escherichia coli (E.coli) by Well cut method. Nutrient agar medium was used to cultivate bacteria. Inoculums (100µL) of fresh cultures were mixed in molten agar medium and poured intopetri dish, then allowed it to solidify. Wells were cut in the media and 0.2 ml formulation F4 (standard), gel with dexamethasone (blank) and pure drug (1.6mg) (control) were added in the wells. After incubation at 37°C for 24hrs the zone of inhibition around the wells was measured.

In vitro **drug release studies of,Dexamethasone and Metronidazole hydrogel**

In vitro drug release studies were performed using vertical modified diffusion tube (surface area: $2cm^2$). 1 ml of gel formulation equivalent to 0.08mg Dexamethasone and 8 mg of,metronidazole was placed in the donor compartment. The receptor compartment consistsof 50ml of phosphate buffer pH 7.2 maintained at 37+0.5°C. The donor compartment was separated from the receptor compartment by prehydrated cellophane membrane. Aliquots(2.5 ml) were withdrawn from the receptor compartment periodically (0.5,1,1.5,2,2.5,3, 3.5hr) and replaced with 2.5 ml of fresh phosphate buffer pH 7.2. After suitable dilution, the sample was analyzed by using shimadzu UV visible spectrophotometer at 320 nm for metronidazole and 217 (λ_1-iso-absorptive point) and 242nm (λ_2- λmax of dexamethasone) for dexamethasone respectively.

In Vivo **study of hydrogel in patients**[15,16,17] **(Specific population)**

Subjects were recruited from patients presenting with oral mucositis to Pain and Palliative Clinic, Medical College , Malabar Area in Kerala.

Method

The study follows a prospective design in which patients are advised to apply hydrogel over the oral mucositis for a period of seven days during which his/her pain status is assessed. Hydrogel was prepared using standard pharmaceutical procedure and is supplied to the patients in 10ml metallic tubes.

Procedure18,19,20

Baseline subjective assessment of pain by the patient on Visual Analog Scale (0-10) and the 5 point pain categorical scale (No pain, Mild pain, Moderate pain, Severe pain, Excruciating pain) was been recorded before the first application of the drug. The first application was done in the presence of a trained nurse. Patient or care giver are advised to apply a thin layer of the preparation evenly on the oral mucositis twice every day and the pain relief status will be recorded by using Visual Analog Scale (0-10).

All patients were provided with a response card on which to record time of application of the gel, pain score on Visual Analog Scale before each application of the preparation and pain relief after second application. Patients were advised to use the preparation twice a day regularly and prorenata doses (p.n.r) in between for 2-7 days and at the end of which they were asked to return the used and unused tubes which are counted as an informal cross check of compliance. All the patients in the study were contacted by one of the investigators every evening for follow up during the study. Patients are questioned about any possible adverse effect during the 72 hours own visit.

The assessments during the study were:

1. Baseline subjective assessment of pain by the patient on Visual Analog Scale before the first application of the study.
2. Patient recording of pain score on the Visual Analog Scale after each application of the medication.
3. The approximate duration of action of hydrogel.

Those patients who reports adequate pain relief after the application of the gel for seven days, are advised to continue the same. Adequate pain relief is considered if there is moderate/total pain relief or a decrease in pain score by 2 points from baseline measurement. The study was stopped once the patient reports inadequate pain relief anytime during the study. Those patients who doesn't respond to hydrogel was discontinued from the study**21,22,23**.

RESULTS AND DISCUSSION

In the preformulation studies, the Dexamethasone and Metroidazole were analysed by IR Spectroscopic method. Results are shown in the figure 1 and 2. The melting point of Dexamethasone and Metronidazole were found to be 252.2^{o}C and 102.2^{o}C respectively. The loss on drying and saturation solubility of both the drugs compiled with that of IP standard. In the compatibility studies, spectrum obtained from the physical mixture of Dexamethasone, Metronidazole, Carbopol940, Xanthan gum were compared with that of pure drugs (Dexamethasone and Metronidazole). (Fig.1,2). All major peaks present in the spectrum of pure drugs were observed in the spectrum of physical mixture of drugs and polymer without change in position. (Fig.3,Table.2).The study clearly indicated the absence of any chemical interaction between the drugs (Dexamethasone and Metronidazole) and the polymers (Carbopol940andXanthangum) and thus confirming that the drugs is compatible with all the excipients used in the formulation (0.5mg/kg b.w/p.o) produced a significant ($p<0.001$) decrease in blood glucose level from first week to third week.

Table 1: Formulation of Dexamethasone and Metronidazole Hydrogel for 100 ml

Ingredients	Formulations				
	F1	F2	F3	F4	F5
Dexamethasone	80 mg	80 mg	80 mg	80 mg	80 mg
Metronidazole	800 mg	800 mg	800 mg	800 mg	800 mg
Carbopol 940	0.5 gm	1 gm	1.5 gm	2 gm	2.5 gm
Glycerine	11 ml	10 ml	9 ml	8 ml	7 ml
Xanthan Gum	20 ml	20 ml	20 ml	20 ml	20 ml
Triethanolamine	0.6 ml	0.6 ml	0.6 ml	0.6 ml	0.6 ml
Distilled Water	q s	q s	q s	q s	q s

Table 2: FTIR Spectroscopic peaks of physical mixture of Dexamethasone, Metronidazole, Carbopol 940 and Xanthan gum

Dexamethasone (cm^{-1})	Metronidazole (cm^{-1})	Drug Excipient Mixture (cm^{-1})	Characterization
1600.16	1360.18	1687.41, 1364.16	Aromatic C=C stretching
1700.23	3453.77	1458.77	Carbonyl stretching
3400	1080.54	3401.82	C-0-C stretching
3020.17	2925.37	2924.52	Olefinic C-H stretching
2850.13	3448.94	3854.04	Aliphatic C-H stretching

Table 3: Viscosity of.the gel formulations. (F1,F2,F3,F4,F5 and F*)

Formulation Code	Viscosity (cps)
F1	60,00,8
F2	103,00,3
F3	125,00,2
F4	157,00,1
F5	161,00,6
F *	167,99,0

Table 4: Extrudability of formulations. (F1,F2,F3,F4,F5)

Formulation Code	Extrudability
F1	++
F2	++
F3	++
F4	++
F5	++

++ = Excellent, +=good values are avg.+SD

Table 5: Drug content uniformity of formulations (F1,F2,F3,F4,F5)

Formulation Code	Drug Content Uniformity(□w/v) For Dexamethasone	Drug Content Uniformity(□w/v) For Metronidazole
F1	90.20±0.713	89.10±0.013
F2	95.16±0.987	93.26±0.537
F3	98.83±1.074	96.91±0.984
F4	100.33±0.884	97.33±0.714
F5	94.80±0.651	95.11±0.218

Table 6: Zone of inhibition of Test formulation, Gel with dexamethasone and pure drug

S. No.	Formulation	Zone of inhibition(cm)
1	Formulation F4 (Test)	1.7
2	Pure drug solution (control)	1.5
3	Gel without metronidazole (blank)	0.3

Table 7: In vitro release study of Metronidazole

Formulation		Time in hours						
		0.5	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**
F1	CDR	2.85± 0.187	3.67± 0.175	4.58± 0.241	5.91± 0.412	6.29± 0.658	6.82± 0.112	7.35± 0.514
	%CDR	35.62± 0.157	45.87± 0.201	57.25± 0.287	73.87± 0.574	78.62± 0.714	85.25± 0.247	91.87± 0.674
F2	CDR	2.68± 0.468	3.06± 0.201	4.36± 0.812	5.65± 0.312	5.98± 0.698	6.71± 0.117	7.23± 0.074
	%CDR	33.5± 0.365	38.25± 0.476	54.58± 0.657	70.62± 0.231	74.75± 0.117	83.87± 0.174	90.35± 0.325
F3	CDR	2.43± 0.256	3.26± 0.258	4.43± 0.467	5.80± 0.219	6.02± 0.439	6.69± 0.987	7.47± 0.951
	%CDR	30.37± 0.141	40.75± 0.245	55.37± 0.657	72.5± 0.124	75.25± 0.572	83.62± 0.168	93.75± 0.175
F4	CDR	3.06± 1.138	3.39± 0.367	4.87± 0.214	5.96± 0.587	6.27± 0.468	6.97± 0.645	7.78± 0.987
	%CDR	38.25± 0.456	42.37± 0.267	60.87± 0.511	74.50± 0.654	78.37± 0.195	87.12± 0.456	97.25± 0.268
F5	CDR	2.86± 0.214	3.68± 0.698	4.48± 0.541	5.68± 0.348	6.18± 0.547	6.73± 0.234	7.61± 0.368

	%CDR	35.75± 0.189	46.10± 0.456	56.78± 0.134	71.12± 0.139	77.25± 0.673	84.12± 0.543	95.12± 0.789

Table 8: In vitro drug release studies of Dexamethasone

Formulation		Time in hours						
		0.5	**1**	**1.5**	**2**	**2.5**	**3**	**3.5**
F1	CDR	0.0215± 0.123	0.0335± 0.897	0.0428± 0.409	0.0571± 0.112	0.0615± 0.678	0.0672± 0.743	0.0725± 0.256
	%CDR	26.87± 0.445	41.87± 0.345	53.50± 0.120	71.37± 0.136	76.87± 0.313	84.25± 0.786	90.62± 0.347
F2	CDR	0.0225± 0.134	0.0318± 0.467	0.0429± 0.233	0.0549± 0.141	0.0601± 0.446	0.0678± 0.567	0.0713± 0.098
	%CDR	28.12± 0.765	39.75± 0.412	53.62± 0.787	68.62± 0.111	75.12± 0.189	84.75± 0.574	89.12± 0.625
F3	CDR	0.0278± 0.316	0.0349± 0.238	0.0413± 0.667	0.0590± 0.119	0.0621± 0.539	0.0689± 0.487	0.0756± 0.651
	%CDR	34.75± 0.241	43.62± 0.445	51.62± 0.157	73.75± 0.824	77.62± 0.712	86.12± 0.178	94.5± 0.237
F4	CDR	0.0309± 1.035	0.0389± 0.879	0.0491± 0.356	0.0593± 0.973	0.0654± 0.256	0.0701± 0.678	0.0789± 0.234
	%CDR	38.62± 0.356	48.62± 0.755	61.37± 0.174	74.12± 0.374	81.75± 0.389	87.62± 0.789	98.62± 0.490
F5	CDR	0.0279± 0.139	0.0357± 0.598	0.0456± 0.441	0.0573± 0.345	0.0610± 0.368	0.0679± 0.214	0.0756± 0.567
	%CDR	34.87± 0.389	44.62± 0.456	57.18± 0.134	71.62± 0.189	76.25± 0.673	84.87± 0.543	94.50± 0.786

Treatment algorithm

If patient with oral mucositis having excruciating pain/difficulty in ingesting food.

Patient enter the study

Assessment of oral mucositis pain using visual analogue scale, Before the application of hydrogel.

Advice to patient/caregiver 

Apply thin layer of hydrogel evenly on the oral mucositis twice a day for 2-7 days.

Refer after 2-7 days

If pain relief is present after 2-7 days.

Stabilize him/her with hydrogel.

Fig. 1. FTIR spectrum of dexamethasone

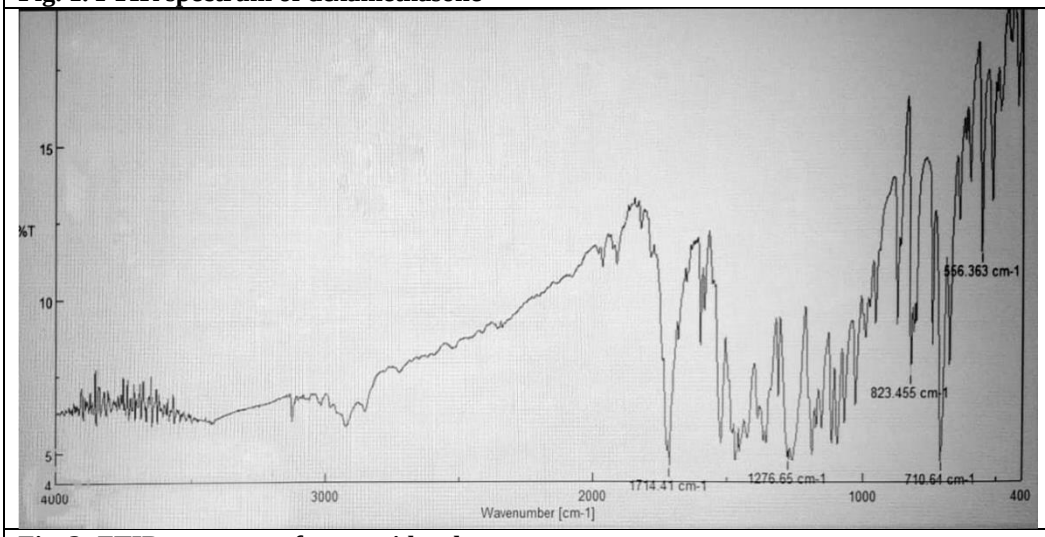


Fig. 2: FTIR spectrum of metronidazole

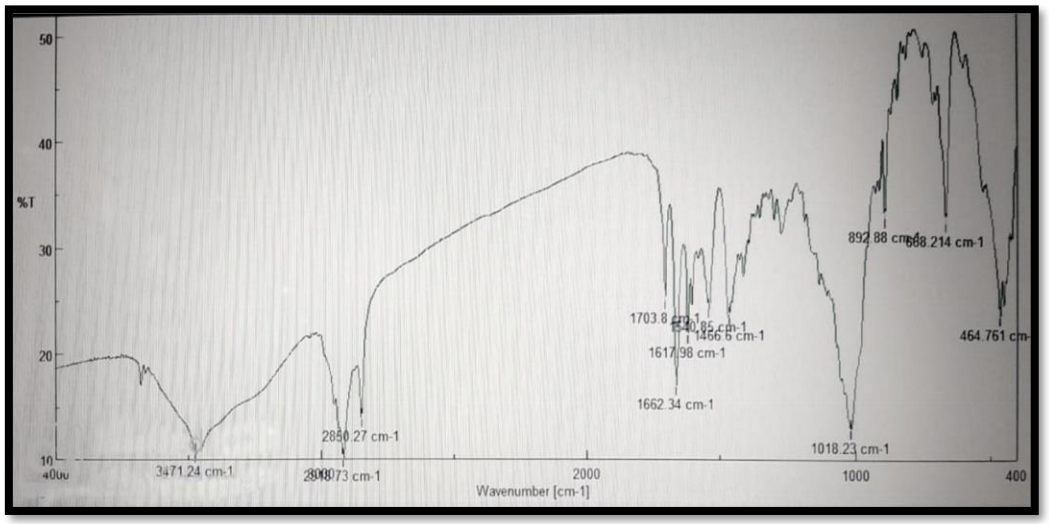


Fig. 3. FTIR Spectrum of the physical mixture of Dexamethasone, Metronidazole, Carbopol 940 and Xanthan gum

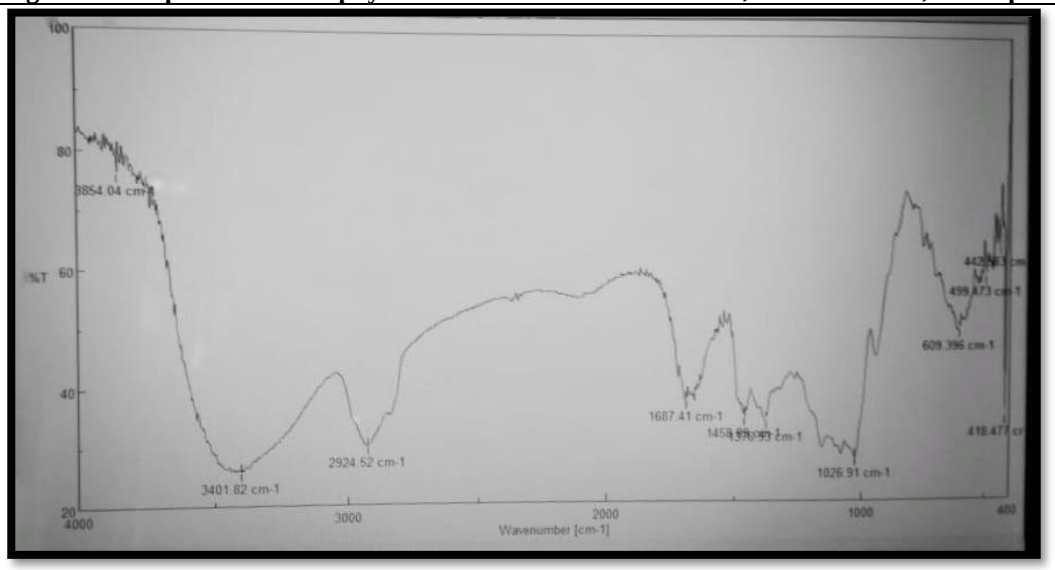


Fig. 4 : DSC of Dexamethasone

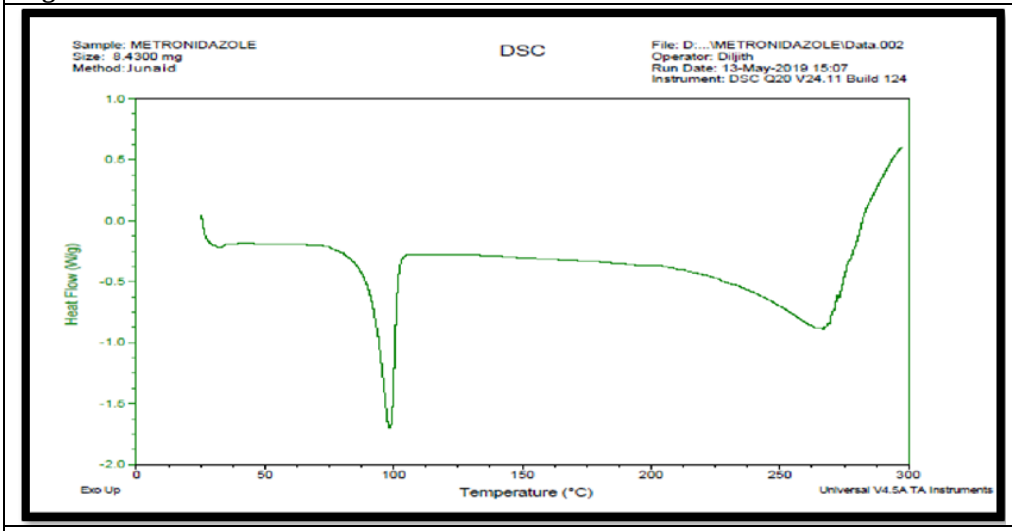
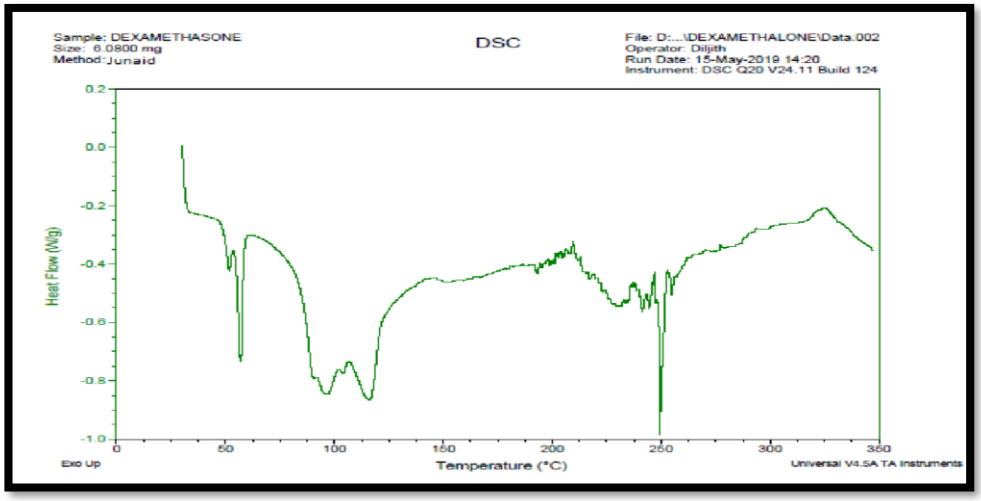
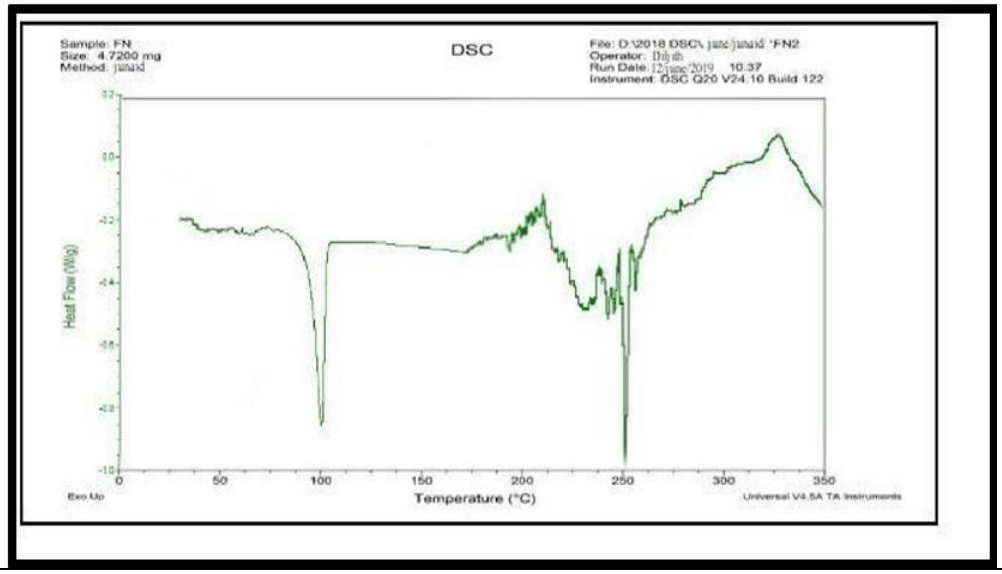


Fig 5:DSC of metronidazole



\\

Fig 6: DSC of mixture



Photographs of chorioallantoicmembrane (CAM) after 5 minutes of application

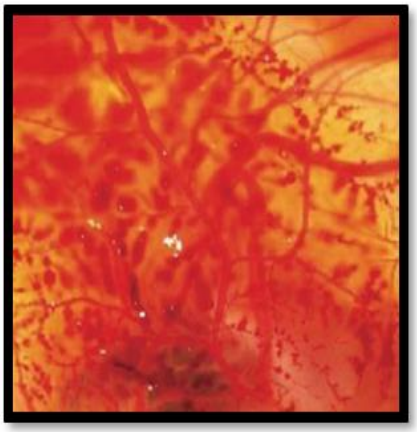


Fig. 7: (Positive control)

Fig. 8: (Negative control)

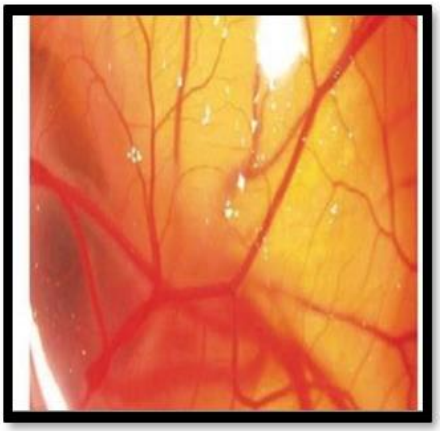


Fig. 9: (Test formulationF4)

Fig. 10 : Zone of inhibition of Test formulation, Gel with dexamethasone and pure drug

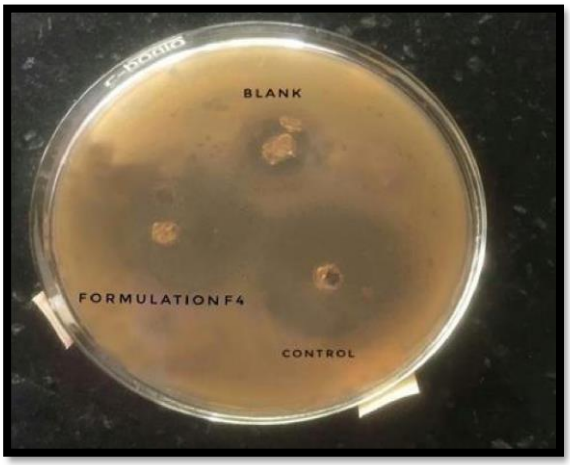


Fig. 11. % CDR Vs Time profile of Metronidazole

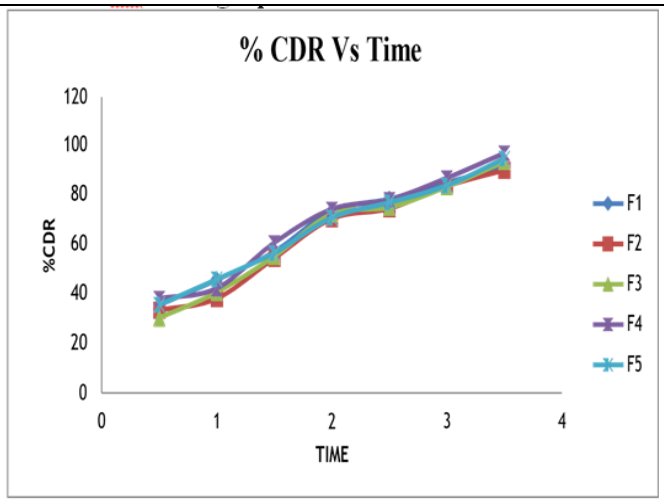


Fig. 12 :% CDR Vs. Time graph of Dexamethasone

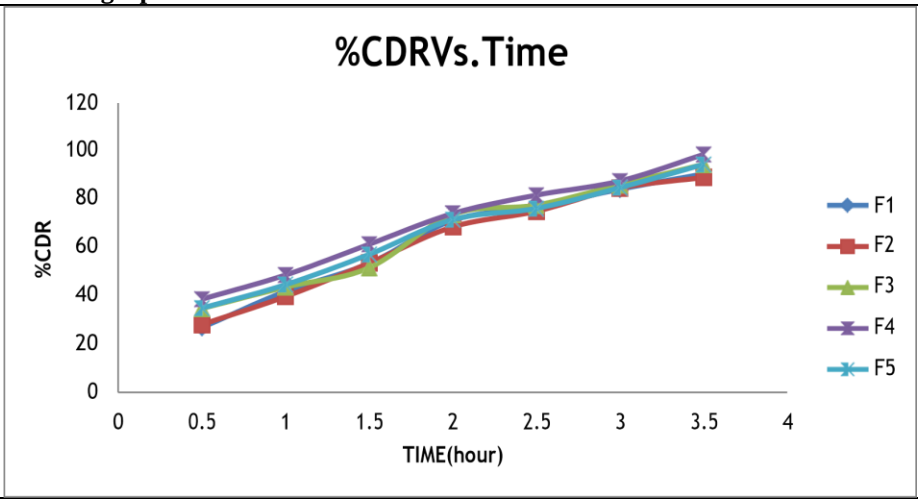
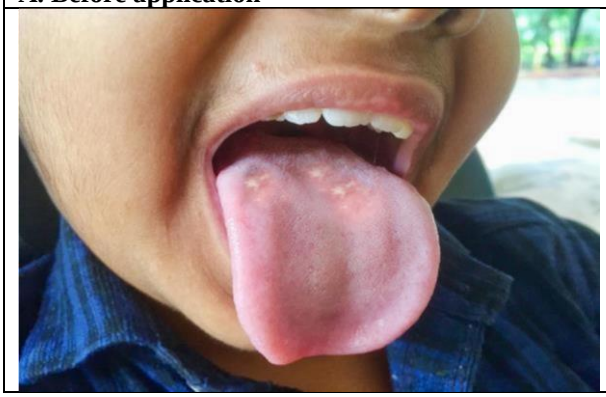
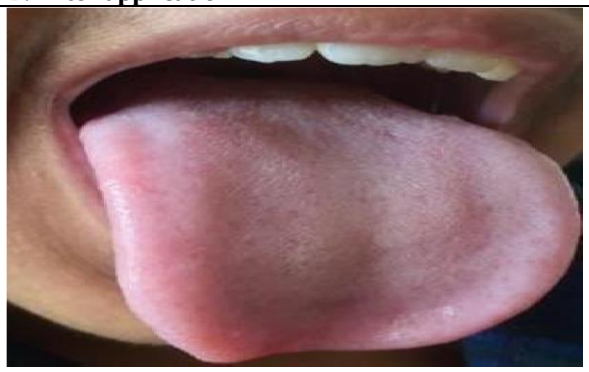


Fig. 13 : Application of the formulated Dexamethasone and metronidazole hydrogel in Oralmucositis patient undergoing radiation/chemotherapy for Osteosarcoma

A. Before application



B. After application



Differential Scanning Calorimetry (DSC)

DSC of drugs separately (Fig. 4, 5) and physical mixture of drugs and the excipients were taken (Fig. 6). The DSC thermogram images of pure dexamethasone shows a sharp peak at 250°C (Fig. 4) and the DSC thermogram images of pure metronidazole shows a sharp peak at 100°C (Fig. 5).The drugs (Dexamethasone and Metronidazole) and excipient mixture was subjected to DSC analysis and the results shows the same characteristics peaks as that of pure drugs (Fig. 6) determining the compatibility of the drugs with excipients.

Measurement of viscosity of the gel

Viscosity of the gel was determined using Brook field viscometer. In order to optimize the viscosity value it was compared with a marketed gel formulation F*.F4 and F5 shows value closer to that of the marketed preparation, it was observed that the viscosity value increased with the increase in the concentration of the Carbopol 940 (Table.3).

Spreadability

The spreadability parameter determines the ease at which the formulation can be applied topically. Spreadability was determined using fabricated wooden block apparatus. It was observed that spreadability decrease with increase in the concentration of thepolymer

Grittiness

All the formulations were evaluated microscopically for the presence of any appreciable particulate matter which was seen under light microscope and was found to good.

Homogeneity

All the five formulation was inspected for the presence of any particulate matter and the appearance and homogeneity was found to be good.

Extrudability

This parameter determines the ease at which the formulation comes out of the metal tube. All the formulations shows good extrudability (Table 4).

Drug Content Uniformity

All the formulation shows excellent drug content uniformity (Table.5). Formulation F4 shows better drug content uniformity compared to other formulations.

HET – CAM Test

No irritation erythema is observed in Test formulation F4 (Fig.9) and Negative control (Fig.7) and erythema is seen only in positive control (Fig.8). So we can conclude that the test formulation F4 was non-irritant and no damage is produced in the CAM.

***In vitro* antibacterial Activity**

In vitro antibacterial activity of gel formulation was performed to evaluate the relative potency of the formulation (Fig. 10, Table. 6).

The study was performed in optimized formulation F4, Gel with dexamethasone, pure drug solution (Fig. 10, Table 6). The optimized formulation (F4) has zone of inhibition of 1.7 Cm and plain drug solution (1.6mg) (control) has zone of inhibition of 1.5 cm and the zone of inhibition of the (blank) gel formulation was found to be 0.3cm. Therefore, we can conclude that the optimized formulation F4 is having better antimicrobial activity 8.7.11 *In vitro* drug release studies of Metronidazole[24]: *In vitro* release study of Metronidazole were performed and the results are given in the (Table. 7). % CDR Vs Time profile of Metronidazole were plotted (Fig. 11).

Diffusion studies of formulations F1,F2,F3,F4,F5 was carried out by using cellophane membrane. 97.25% of drug diffusion was obtained at 360 minutes for formulation F4 (Table. 7). F4 formulation showed the maximum release of drug after 30 minutes of

release study, it is very important in order to produce therapeutic response (Fig. 11). *In vitro* release studies of dexamethasone were performed and the results are given in the (Table. 8). Diffusion studies of formulations F1,F2,F3,F4,F5was carried out by using cellophane membrane. 97.25% of drug diffusion was obtained at 360 minutes for formulation F4 (Table 8). F4 formulation showed the maximum release of drug after 30 minutes of release study, it is very important in order to produce therapeutic response (Fig.12).

CONCLUSION

The goal of present study was to convert an extemporaneous preparation 'Gudallurmix' into a gel formulation. In order to reduce the pain and suffering of oral mucositis patients who are undergoing chemotherapy/radiationtherapy. This dissertation work mainly focus on the use of dexamethasone and metronidazole as a potential candidate for the management of oral mucositis. Gel formulation usinghydrophilic polymers were preferred in order to produce a rapid release of drug from the gel formulation. Spreadability and extrudability of all formulations was maintained well. *In vitro* irritation test (HET – CAM) were carried out by using the optimized formulation F4. F4 was found to be non-irritant. Antimicrobial activity of metronidazole was studied using agar diffusion method on the culture plates of E. coli. F4 shows antimicrobial activity against E.coli. *Invitro* release study was done inorder to optimize the formulations. Isoabsorptivepoint method is employed for the simultaneous estimation of dexamethasone and metronidazole. F4 showed maximum of 97.25% of drug release for dexamethasone and 98.62% of drug release for metronidazole, Also with maximum initial release to produce rapid analgesic action. *In vivo* patient study where done with optimized formulation F4. Painful oral mucositis conditions associated with carcinoma Buccalmucosa, Oral cancer, ulcerative lesions etc showed encouraging analgesic effect and subside the other discomforts of the patients with dexamethasone and metronidazole hydrogel therapy. Currently the management of oral mucositis is by the aid of oral steroids and various mouth paints of (clotrimazole, Benzalkonium chloride, Triamcinolone) which are known for severe adverse effects and produce a burning sensation while applying over the oral mucosa and tongue. This burning sensation and other irritations can be reduced by the dexamethasone and metronidazole hydrogel. Pain and other discomfort relief was reported in every patients. Pain is reduced by 75-100% and thus the application of dexamethasone and metronidazole gel greatly reducing the use of oral steroids and other mouthpaints and thus also limiting the severe adverse events. Hydrogel study is still going on in institute of pain and palliative medicine. Dexamethasone and Metronidazole hydrogel can be prepared at a rate of Rs45/-for 10 ml tube, which is less than the price of other preparations available in the market used in the management of oral mucositis. Thus, the hydrogel formulation developed has proven to be a 'boon' to a population who severely suffers a lot of pain in their lives.

REFERENCES

1. Siju E.N., Minil M, Rahul K. Study of in vitro antioxidant, anti-inflammatory and anticancer potential of *Blumea oxyodonta* dc 2019;11(1) 9-19.
2. Siju E. N., G. R. Rajalakshmi, Anu P. Paulose. CADD: Pharmacological approaches in drug design and drug discovery 2017;6(4) 892-908.
3. Jones JA, Avritscher EB, Cooksley CD, et al. Epidemiology of treatment associated mucosal injury after treatment with newer regimens for lymphoma, breast, lung, or colorectal cancer. Support Care Cancer 2006; 14 (6):505–15.
4. Vera-Llonch M, Oster G, Ford CM, et al. Oral mucositis and outcomes of allogeneic hematopoietic stem-cell transplantation in patients with hematologic malignancies. Support Care Cancer 2007; 15 (5):491–6.
5. Managing oral mucositis in patients with hematologic malignancies. J Support Oncol 2006;4(2):79.
6. Anand B. Application of organogels in pharmaceuticals. Journal of Science and IndustrialResearch,2001;60(4):311-18.
7. Penzes T, Blazzo G, Aigner Z, Falkay G, Eros I. Topical absorption from organogels- invitro and invivo correlations International Joumal of Pharmaceutics 2005,208:47- 54. 7.
8. Piyush Gupta and Sanjay Garg. Recent advances in Semisolid dosage forms for dermatological application. Pharmaceutical Technology 2002 March:144-62.
9. Joel.L.Zatz and Gregory Kushal P. Gels. In pharmaceutical dosage forms disperse system Liberman, Reiger and Banker, Published by Marcel Dekkar Inc, NewYork vol. 2: 495-508.
10. Ferrari F. et al description and validation of an apparatus for gel strength measurements. International].Pharm-1944:109(2):115-124
11. CristinaL,ChivaC,HerdaR,Corrisitex BCOP and HET-CAM as alternative methods to animal experimentation, Braz J Pharm Sci,2009:44
12. Barry B W, Mayer M C "The rheological property of carbopolgel-1 continuous shear and creep properties of carbopol gels" indjpharm1979.1-2.

13. Soohi Patel, Chandrasekhar Garapati,. Development of delivery strategy for topical application of dexamethasone for posterior uveitis therapy.IntJ Pharm,2008;165:33- 44.
14. AneesFathima, AmreenFathima, McGilveray, I. J.,Sved, S. Systemic absorption of metronidazole by the vaginal route. Obstet. Gynecol. 2017, 65: 781–784.
15. Hanna, M.H., Elliott, K.M., 2005. Randomized, double-blind study of the analgesic efficacy of morphine-6-glucuronide versus morphine sulfate for prospective pain in major surgery. Anesthesiology 102,815-821.
16. Patel MM., Sheth MN., and Hariyanawala Al., "Comparative studies in the release profile of Metoprololtartarate from transdermal gel using human and animal skins", The Eastern Pharmacist, 2009 Apr;137-138.
17. Khalil-A1-Khamis., Stainley S., Davis., Jonathan Hadgraft., "In vitro in vivo correlations for the percutaneous absorption of Salicylates", Int. J. Pharm., 1987; 40:111-118
18. Behl,H Char,S.B Patel, B.Metha,D.Piemontese and A.W.Malick In vivo and in vitro skin uptake and permeation studies: critical considerations and factors which affect them. In V. P. Shah and H. I. Maibach (eds.), Topical Drug Bioavailability, Bioequivalence, and Penetration, Plenum,New York, 1993, pp.225–252.
19. Patel MM., Sheth., and Hariyanawala Al., "Comparative studies in the release profile of Metoprololtartarate from transdermal gel using human and animal skins", The East Pharmacist, 1995 Apr;137-138.
20. Cunningham, F. E., Kraus, D. M., Brubaker, L., Fischer, J. H. Pharmacokinetics of intravaginalmetronidazole gel. J .Clin. Pharmacol. 2007, 34:1060–1065
21. Raber-Durlacher JE, von Bultzingslowen I, Logan RM, et al. Systematic review of cytokines and growth factors for the management of oral mucositis in cancer patients. Support Care Cancer2013;21(1):343–55.
22. Duncan GG, Epstein JB, Tu D, et al. Quality of life, mucositis, and xerostomia from radiotherapy for head and neck cancers are port from the NCICCTGHN2randomized trial of an antimicrobial lozenge to prevent mucositis. Head Neck 2005;27(5):421–8.
23. Gondaliya D.P PanderiKakshudu K studies on permeation characterization and transdermal permeation of Nimesulide from aqueous and emulgel. Indian drugs 2002; 39(9) 465-73
24. Seth P.L. Percutaneous absorption of lbuprofen from different formulations, Arzneimittel Forschmug,1993:43(8):919-21.
25. Rama Rao., Srinivas V., Prakash V., Diwan., "Influence of permeation enhancers on the in-vivo percutaneous absorption of indomethacin", The Eastern Pharmacist, 1997 Aug; 155-1580.