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Article

A comparative physicochemical and pharmacological evaluation of dexamethasone sodium phosphate and betamethasone sodium phosphate mucoadhesive gels for the treatment of oral submucous fibrosis in rats

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The present study is aimed to formulate steroidal oral mucoadhesive gels of dexamethasone sodium phosphate and betamethasone sodium phosphate. Six gel formulations each of dexamethasone sodium phosphate and betamethasone sodium phosphate prepared using two different polymers carboxymethyl cellulose sodium and hydroxypropyl methylcellulose, in variable proportions. All the formulations subjected for assessment of various physicochemical parameters and mechanical properties. The formulations BSP_5 and DSP_5 both containing 1.25 % carboxymethyl cellulose sodium, 1.25 % hydroxypropyl methylcellulose, exhibiting mucoadhesive strength of 12.300 ± 0.004 and 12.600 ± 0.01, adhesiveness of 28.04 ± 00 and 30.02 ± 00, cohesiveness of 28.04 ± 00 and 30.02 ± 00, drug release of 86.869 ± 0.380 % and 88.473 ± 0.457 % respectively were considered as promising ones and were further subjected for stability studies and *in vivo* study in male albino rats. Formulation DSP_5 upon oral application for 4 months in arecoline induced oral submucous fibrosis rats, showed more than 80 % reduction in fibrosis as compared with BSP_5 which showed nearly 50 % reduction. These results were concluded on the basis of histopathological profile and weight gain among the experimental animals during *in vivo* study. Hence, DSP_5 by minimizing the painful injuries and morbidities justifies being suitable noninvasive model for OSMF treatment.

Keywords: Oral submucous fibrosis (OSMF). Carboyxmethyl cellulose sodium (Na CMC). Hydroxypropyl methyl cellulose (HPMC). Dexamethasone sodium phosphate (DSP). Betamethasone sodium phosphate (BSP).

INTRODUCTION

Betamethasone sodium phosphate and dexamethasone sodium phosphate are disodium phosphate salts of their respective 21-phosphate esters (British Pharmacopoeia, 2008). Both the drugs have immunosuppressive, anti-inflammatory and anti-fibrolytic action (Mastan *et al.*, 2013). These are therefore used to treat oral submucous fibrosis. Oral submucous fibrosis (OSMF) is an intensive precancerous condition connected to the mouth, esophagus and pharynx characterized by hyalinized oral connective tissue and atropic epithelium (Pillai, Balaram, Reddiar, 1992; Sores, Perschbacher, Ordonez, 2018; Pindborg, Sirsat, 1966). OSMF occurs due to excessive chewing of areca nut in various forms such as gutkha, pan masala, betel quid (Arakeri, Brennan, 2013; Angadi, Ramsay, 2011; Nair, Bartsch, Nair, 2004). The risk of OSMF is more with frequent and chronic chewing habit (Sinor *et al*., 1990; Tilakaratne, *et al.*, 2006). The phytoconstituents of arecanut, in other words. - Arecolines, catechins and tannins are known to activate the β-transforming growth factor that increases collagen production and causes abnormally excess collagen deposition in oral submucosa (Rajalalitha, Vali, 2005; More *et al*., 2015). Therefore, OSMF is related to non elasticity

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and development of vertical fibers in labial and oral tissues (Ali, Patil, Hosant, 2014). All these alterations eventually lead to a progressive restriction of mouth opening, difficulty in tongue protrusion, difficulty in chewing and swallowing processes. Inflammation, hypovascularity, ulceration, blanching and burning sensation are the other symptoms and signs of OSMF (Wahi, Luthra, Kapur, 1966; Gupta, Srinivasan, Daniel, 2012; Gupta, Sharma, 1988). Treatment regimes such as intralesional injections of placental extract and hyaluronidase, both individually combined with dexamethasone were found to decrease the burning sensation and increase the mouth opening in OSMF patients (Shah PH, Venkatesh R, More CB, Vassandacoumara V, 2016; James *et al*., 2015). Likewise, intralesional betamethasone injection had also been found to be effective in OSMF management (Singh, *et al*., 2014). These traditional OSMF therapies with submucosal corticosteroid injections, lead to irresistible pain, bleeding, scaring, bruising, infection, disrupted wound healing, exacerbated fibrosis and marked trismus in oral cavity (Thakur, Keluskar, Bagewadi, 2011). Thus the most demanding alternative treatment approaches are retentive oral muco-adhesive formulations, such as pills, films, patches, ointments, pastes and gels, since these are non-invasive, low traumatic and self-applicable among OSMF patients (Yajaman, Ketousetuo, Bandyopdhyay, 2006). Dexamethasone sodium phosphate mucoadhesive buccal patch prepared by solvent casting technique with chitosan as polymer, PEG 400 as plasticizer, ethyl cellulose and isopropyl alcohol as supporting membrane (Polshettiwar et al., 2019) and betamethasone sodium phosphate mucoadhesive buccal patch prepared by solvent casting technique with HPMC E5 LV and carbopol 9409 as polymer, PEG 1000 as plasticizer were effective in overcoming of the side effects of injection and also ensured adequate levels of oral drug release during OSMF treatment. For effective management of many oral mouth lesions, oral bioadhesive corticosteroid gels were also employed with appropriate viscosity, spreading and release properties. Mucoadhesive oral gels for triamcinolone acetonide were prepared employing carbopol 934, chitosan and HPMC in order to increase adhesive performance and to promote easy application (Amasya, *et al*., 2012). Triamcinolone acetonide oro mucoadhesive paste was prepared using plastibase (95 % mineral oil and 5 % polyethylene) as well as various ratios of hydrocolloid solids for the treatment of aphthous stomatitis. The formulation containing plastibase (60 %), pectin (3.3 %) and gelatin (30 %) was stated to exhibit strong adhesion, spreadability and rheological properties (Hamishehkar, *et al*., 2015).

In this regard, a novel strategy for the formulation and evaluation of Dexamethasone Sodium Phosphate (DSP) and Betamethasone Sodium Phosphate (BSP) glycerine-based mucoadhesive gel incorporating synthetic hydrophilic mucoadhesive polymers such as sodium CMC, HPMC K100M, alone and in combined concentrations was therefore envisaged in this report. These polymers undergo a phase shift from liquid to semisolid when applied to oral mucosa to promote intimate interaction with the underlying absorption surface (Ahuja, Khar, Ali, 1997). The gel form increases viscosity leading to a continuous, regulated and targeted delivery of drugs for better care and immediate onset of action leading to greater patient conformity (Boddupalli, 2010).

The prepared gel formulations were intended to evaluate for physical-chemical properties in order to select DSP and BSP promising formulation, optimized formulations were further aimed at pharmacologically screening for their therapeutic efficiency in OSMF induced male albino rats and histopathological studies. The optimized formulation that effectively reduced fibrosis in OSMF induced rats during treatment was subjected to cytotoxicity, as no cytotoxicity studies were ever reported involving these drug formulations for their application in OSMF treatment. Therefore the present study stands out to be novel and unique both in the formulation development and toxicity reporting.

MATERIAL AND METHODS

Material

Betamethasone sodium phosphate was obtained as a gift sample from Glenmark Pharmaceuticals Ltd**.** **(**Malegaon, India), dexamethasone sodium phosphate was a gift sample from IPCA Laboratories Ltd. (Vapi, India), hydroxylpropyl methyl cellulose (HPMC K100) was purchased from N R chemicals (Mumbai, India), carboxy methylcellulose sodium, (Na-CMC- High viscosity grade

1100- 1900 cp), sodium metabisulphite and glycerin were purchased from S.D. Fine Chemicals (Mumbai, India).

Formulation of oral mucoadhesive BSP and DSP gels

HPMC and Na-CMC were used alone in conjunction with appropriate quantity for the preparation of gels (Chen, Yan, Shuying, 2018; Yan *et al*., 2017). Table I demonstrate the composition of gel formulations with various polymers. Previously dissolved drug in glycerin was combined with polymers, sodium metabisulfite and required 24 hours to be hydrated. The ready gels were filled and labeled in empty aluminum tubes (Bhatia, Sachan, Bhandari, 2013).

TABLE I - Formulation of oral mucoadhesive gels

Gel code	Drug (g)	Na CMC (g)	HPMC (g)	Sodium meta Bisulphite (mg)	Glycerin (ml)
BSP_1	0.1	2.5	_	0.01	100
BSP_2	0.1	3.0	_	0.01	100
BSP_3	0.1	_	2.5	0.01	100
BSP_4	0.1	_	3.0	0.01	100
BSP_5	0.1	1.25	1.25	0.01	100
BSP_6	0.1	1.50	1.50	0.01	100
DSP_1	0.1	2.5	_	0.01	100
DSP_2	0.1	3.0	_	0.01	100
DSP_3	0.1	_	2.5	0.01	100
DSP_4	0.1	_	3.0	0.01	100
DSP_5	0.1	1.25	1.25	0.01	100
DSP_6	0.1	1.50	1.50	0.01	100

Na CMC = Sodium carboxy methyl cellulose HPMC = Hydroxy propyl methyl cellulose

Quality control of prepared oral mucoadhesive gels

Homogeneity

Formulated gels were allowed to be settled in a clean glass beaker and analyzed for proper appearance and presence of aggregates (Tanwar, Jain, 2012).

Grittiness

For some particulate matter, all gel formulations were microscopically evaluated (Tanwar, Jain, 2012).

Spreadability

Between two horizontal plates (20 cm x 20 cm), one gram of prepared gel was placed to which a load of 125 g was applied above the top plating. After 1 min, the gel spread diameter was measured (Tanwar, Jain, 2012).

Extrudability.

One-ounce collapsible tube with 5 mm nasal opening was filled with a prepared gel. The extrudability of the gel was measured by calculating the volume of gel extruded through the tip while adding a constant weight of 1 kg. The extruded gel was collected and weighed on the pan (Tanwar, Jain, 2012).

pH measurement

Approximately 5 g gel dissolved in 45 ml water, the pH of this suspension was measured at 27 °C using the pH meter (pH ep@ - pocket size pH meter, model no. S221504, Hanna Instruments, Italy) (Tanwar, Jain, 2012).

Drug Content Uniformity

One gram of gel containing 1000 µg of DSP and BSP; each dissolved separately in 100 mL of phosphate buffer solution of 6.4 pH to give a concentration of 10 mcg/mL. The absorbance was assessed at 242 nm for DSP gel and at 240 nm for BSP gel respectively using U.V.Spectrophotometer (Shimadzu UV Spectrometer, model 1800 240V, Japan) against blank. The blank solution was prepared in the similar manner as above using gel containing respective polymers and other additives without drug (Tanwar, Jain, 2012).

Viscosity

Brookfield Capcalc V3.0 Build 20.0 viscometer was used to analyze the viscosity of gels with spindle-01. Measurements were recorded at speed varying from 10 to 30 rpm at 30 s between two successive speeds as equilibrium time and then in a descending order, with a shear rate of 133 to 400 s^{-1} (Manavalan, Ramsay, 2006).

The viscosity data were plotted as

1. Shear rate versus shear stress.
2. Log of shear rate versus log of shear stress.
3. Viscosity versus speed.

Mucoadhesive study

The bioadhesive force of the prepared gels was calculated by the use of the assembled system developed in our laboratory. Sections of the fresh goat oral tissue were fixed with cyanoacrylate adhesive, enabling the mucosal surface outside on two glass vials. One vial was attached to the balance; the other vial was mounted on a height-adjustable pan. Around 1 g gel was added to a vial's buccal tissue. The height of the other vial was subsequently modified such that the gel applied on one vial's mucosal surface could coincide and bind vertically to the mucosal surface of the other. The weight was raised progressively until the two vials eventually became separated. The bioadhesive force of the gel under analysis was calculated on the basis of the minimum weights required for the two vials to be separated. For each measurement, the pieces of oral tissue were altered. All the above studies were performed in triplicate (Suresh, Manhar, 2014).

Texture Profile Analysis

Texture profile analysis was used to quantify different mechanical characteristics of prepared gels using Texture Profile Analyzer (Texture Pro CT V1.8 Build 31, Brookfield Engineering Labs, USA). The gel under examination was transferred into a beaker. The analysis was carried out by compressing the gel sample twice using an analytical probe (10 mm diameter) at a rate of 2 mm/sec to a depth of 15 mm, with a delay of 15 s between the end of the first compression and the beginning of the second one. The force to trigger was 3 g. All assessment was done in triplicate. The resulting force-time plots-and force-distance plots were computed to define the following mechanical parameters (Jones, Woolfson, Brown, 1996)

1. Hardness- The total force for deformation assessment.

2. Compression- The work essential to compress the formulation during the probes first pass.
3. Adhesiveness- The work required to combat attractive forces between the probe/sample surfaces
1. Cohesiveness- The work required for the sample surface to be united with the surface of the probe.

In vitro diffusion profile

Two glass cylinders open at both ends were used for the drug diffusion analysis. Each cylinder was 10 cm in height with an external diameter of 3.7 cm and an inner diameter of 3.1 cm. One end of each cylinder was covered with a dialysis membrane – 70 (Hi-Media) to obtain two permeating cells. One gram of DSP gel was inserted within one permeation cell while another permeation cell contained 1 g of BSP gel. Two beakers, containing 100 mL of 6.4 pH buffer solution, served as receptor compartments for each permeation cell. The sample was immersed in the receptor compartment exactly beneath the surface of the buffer medium. Using a magnetic stirrer at 37 °C, the media in each receptor compartment was agitated at a rate of 50 rpm. After every 10 min of time interval, 10 mL samples were removed and assayed using a UV spectrophotometer (Shimadzu UV Spectrometer, model no. 1800 240V, Japan) at 242 nm and 240 nm respectively for DSP and BSP gel. After each sampling, the aliquots were replaced by the same quantity of fresh medium. All the experiments were carried out in triplicate (Patel, Gandkar, Soudagar, 2013).

The results of *in-vitro* release were fitted into four models of data treatment as follows.

1. Percent cumulative drug release versus time.
2. Log percent cumulative drug remaining versus time.
3. Percent cumulative drug release versus square root of time.
4. Log percent cumulative drug release versus log of time.

Fourier Transform Infrared studies (FTIR)

The FTIR experiments were carried out using the Infra-red spectrophotometer (Shimadzu, Model Alpha E, FTIR Burker Germany). In order to determine all drug-polymer interactions, the spectrum of pure drug and gel formulations were investigated.

Stability studies of oral gel formulations

Stability analysis was performed for DSP and BSP's most appropriate formulation. The gel under analysis was sealed in a glass vial and held for 6 months in electronic desiccators (Bel – Art Dry – Keeper PVC Vertical Auto – Desiccator Cabinet, Model No. 420561003, NJ-USA) at 30 ± 2 °C, relative humidity 65 ± 5 and 40 ± 2 °C, relative humidity 75 ± 5. The sealed glass vials containing gel samples were also preserved for the same time in the refrigerator at 4 ± 1 °C. In the first, third and sixth months, the gel samples were obtained in order to assess alterations in physical appearance, pH, drug level uniformity, mucoadhesive strength, viscosity and mechanical properties due to temperature and humidity (Kumar, Verma, 2010).

In vivo studies for evaluation of reduction in fibrosis in arecoline-induced OSMF rats

In-vivo research used male Wistar albino rats of 240 g to 250 g body weight. Approval of the study protocol prior to the initiation of animal testing was obtained from the Institutional Animal Ethical Committee (HKES/MTRIPS/IAEC/93/2017-18) in compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA). The experimental animals were divided into 4 groups, consisting of 9 rats in each group. Group I was normal control (G-I). Group II was OSMF induced group (G-II). Group III included OSMF induced rats treated with DSP gel (G-III) and group IV included OSMF induced rats treated with BSP gel (G-IV).

The *in vivo* study was carried in two phases (Wen *et al*., 2017; Zhang *et al*., 2016).

Induction of OSMF in rats

In healthy rats, Arecoline hydrobromide mucoadhesive gel (Table II) was used to induce OSMF by bilateral

application to the buccal mucosa twice daily for 4 months with the aid of cotton bud. After the gel was applied, the animals were fasted for 6 hours and then fed a standard diet*ad libitum*. At the end of 1st, 2nd and 4th month respectively, one rat from each group with lowest body weight was sacrificed and the biopsy was collected from oral mucosa using skin biopsy punch No. 3.5. For histopathological studies, the biopsies were stored in normal saline vials.

TABLE II - Formulation of oral mucoadhesive arecoline gel

Ingredients	Quantity
Arecoline hydrobromide	1.00 g
Carboxymethyl cellulose sodium	1.25 g
Hydroxypropyl methylcellulose	1.25 g
Sodium metabisulphite	0.01 mg
Water	100 ml

Treatment of OSMF induced rats

The optimized gel formulation DSP_5 and BSP_5 respectively were processed for animals induced by OSMF. For 4 months, the gel was added every day to the OSMF mediated mucosa of the rats. Two rats from Group III & IV, each were killed and oral mucosal biopsies were taken on histopathological assessments at the end of the 1st, 2nd and 4th months after continual gel applications.

The oral mucous mucosa samples from group I and II were obtained and preserved for comparison.

In-vitro cytotoxicity study

Buccal tissues obtained from anesthetized normal healthy rats and OSMF induced rats by incisional biopsy were minced into 1 mm × 1 mm × 1 mm pieces, washed in Dulbecco's phosphate buffer saline (DPBS) and subjected for incubation for 18 h in working media (Dulbecco's Modified Eagle Media) added with 10 % Fetal bovine serum, 100 µg/mL of penicillin, 100 µg/mL of streptomycin and 1 µg/mL of amphotercin B containing crude collagenase followed by centrifugation at 3000 rpm for 3 min. The sediment was spread for 48 h on a 60 mm tissue culture plate containing working media to promote cell attachment. For every 3rd day, the media was substituted for fresh one. Confluence was attained in 2–3 weeks, after which the cells were subcultured. Humidified atmosphere of 95 % air, 5 % CO_2 and 37 °C was provided for maintenance of the cultures. The fourth passage cells were selected for the study.

The MTT reduction and LDH release assays were performed on 96-well plates (2 x 10^5 cells per mL) of a complete 100 µL growth medium using the normal and OSMF-induced cells. The cells at a density of 2 × 10^5 cells/mL were exposed to the drug concentrations at 1mM, 10 mM and 100 mM respectively and incubated for 24 h. Later MTT reduction and LDH release assays were performed (Surampalli, Nanjwade, Patil, 2015).

MTT reduction assay

At a density of 2 x10^5 cells/mL, normal and OSMF-induced cells were exposed to drug concentrations of 1 mM, 10 mM and 100 mM respectively in triplicates in 96-well plates and incubated at 37 °C for 24 hours. 20 µL of MTT (5 mg/mL PBS) was added to each well after incubation, and 200 µL of DMSO was added to all wells and thoroughly mixed. The plates were read on the Micro-Elisa reader at 570 nm. The viability of the OSMF cells was determined on the basis of spectrophotometric measurements relative to the control cells (control cell absorbance as 100 % viability). Cell viability was measured as percent of dead cells = 100-(OD treated/OD control) × 100.

LDH release assay

The commercially available LDH release assay kit (Sigma-Aldrich, India) was used. Triplicate assay was performed. The percentage of LDH released was determined as follows: percent of the LDH released = (R LDH absorbance/T LDH absorbance) × 100 percent.

Statistical studies

The findings were expressed as mean ± SD and data analysis using Graph Pad Prism 5 software (Graph Pad, San Diego, CA, USA) using one-way variance analysis (ANOVA) followed by Dunnett's or Bonferroni post-test for multiple comparisons with $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Homogeneity

According to the results of the analysis, all prepared gel appearances were smooth and free of lumps and grittness. All gel formulations exhibited uniformity of the drug content and no particulate matter was observed under the light microscope (Table III) (Aslani, Melekpour, 2016).

TABLE III - Evaluation parameters for oral mucoadhesive gel formulations

Formulation	Homogeneity	Grittiness	Viscosity at low shear rate (cps)	Viscosity at high shear rate (cps)	Spreading diameter after 1 min* (mm)	Extrudability	Mucoadhesive Strength* (g)	pH*	Drug content uniformity* (%)
BSP_1	+++	-	11355	5252	39±3.0	+++	13.700±0.004	6.7±0.55	98.96±0.208
BSP_2	+++	-	12788	6997	27±2.0	++	13.850±0.003	6.6±0.35	98.53±0.185
BSP_3	+++	-	10104	4469	55±1.2	+++	13.100±0.006	6.5±0.40	99.45±0.180
BSP_4	+++	-	10987	4833	51±0.8	+++	13.400±0.005	6.9±0.10	99.21±0.110
BSP_5	+++	-	8420	3670	63±1.0	+++	12.300±0.004	6.4±0.30	99.94±0.211
BSP_6	+++	-	9020	3706	60±1.5	+++	12.600±0.005	6.5±0.15	99.63±0.550
DSP_1	+++	-	11550	5412	37±2.1	+++	13.700±0.003	6.5±0.26	97.60±0.299
DSP_2	+++	-	12991	7087	26±2.0	++	13.900±0.05	6.4±0.20	97.00±0.599
DSP_3	+++	-	10958	5068	51±0.6	+++	13.400±0.03	6.4±0.25	98.20±0.598
DSP_4	+++	-	11404	5599	38±3.2	+++	13.700±0.005	6.7±0.21	97.90±0.346
DSP_5	+++	-	8976	4083	60±2.1	+++	12.600±0.01	6.4±0.20	98.80±0.623
DSP_6	+++	-	9728	4509	56±1.0	+++	13.000±0.07	6.4±0.25	98.50±0.173

+ = Poor, ++ = Fair, +++ = Good * Values mentioned are the average of three determinations

Viscosity

The gel viscosity depended directly on the formulations polymeric concentration. The prepared gels viscosity increased with increased polymer concentration due to increased internal friction between randomly coiled, swollen polymer molecules and solvent molecules around them.

The measurements of viscosity were performed at varying speed and shear rates. The viscosity of BSP gels ranged from 8420 ± 8 to 12788 ± 9 cp and 3670 ± 4 to 6997 ± 2 cp, at low and high shear rates respectively. The viscosities of DSP gels at low and high shear rates ranged from 8976 ± 10 to 12991 ± 9cp and 4083 ± 3 to 7087 ± 2cp respectively (Table III). No statistically significant difference was noticed between the viscosities of BSP and DSP gels at $p > 0.05$.

The DSP_2 and BSP_2 gels prepared using 3 % Na CMC had highest viscosity of 12991 cp and 12788 cp. The gel DSP_4 and BSP_4 prepared using 3 % HPMC displayed viscosity lower than the gels prepared using Na CMC (11404 cp and 10987 cp respectively), while the gels DSP_5 and BSP_5 prepared using a mixture of 1.25 % Na CMC and 1.25 % HPMC had the lowest viscosity of 8976 cp and 8420 cp respectively (Shah, Mehta, Patel, 2011).

A straight line was obtained with slope N when plotting log of shear stress versus log of shear rate (Figure 1A and 1B). The N values were 3.348, 2.216, 3.847, 3.957, 4.109 and 5.166 for BSP_1, BSP_2, BSP_3, BSP_4, BSP_5 and BSP_6 respectively, while the N values were 3.270, 2.194, 3.335, 2.794, 4.563 and 3.35 for DSP_1, DSP_2, DSP_3, DSP_4, DSP_5 and DSP_6 respectively (Manavalan, Ramasamy, 2006).

The plots shear rates at different shear stresses in all gel formulations were obtained with a clearly distinguished 'up' and 'down' curve (Figures 1C and 1D) (Manavalan, Ramasamy, 2006).

.The viscosity-to-speed graph was plotted indicating that the viscosity of the gels was inversely proportional to the speed. The curve appeared as a straight line at higher speed and displayed the lowest viscosity of the formulations (see Figures 2A and 2B) (Manavalan, Ramasamy, 2006).

The viscosity results showed the shear thinning/ pseudoplastic behavior of the BSP and DSP gel formulations at room temperature, whereby the viscosity of gels decreases with increasing spindle speed or shear rate (Figures 2A and 2B). The slope N of straight line obtained in the plot of log of shear stress versus log of shear rate was greater than 1 for all the prepared gels with slope values of formulations of DSP_5 and BSP_5 as 4.563 and 4.109 respectively. The N value should be greater than 1 for the pseudoplastic property (higher N value means better pseudoplastic behavior) and the 'up' and 'down' rheogram curves obtained in the plot of shear rates versus shear stresses should not superimpose each other.

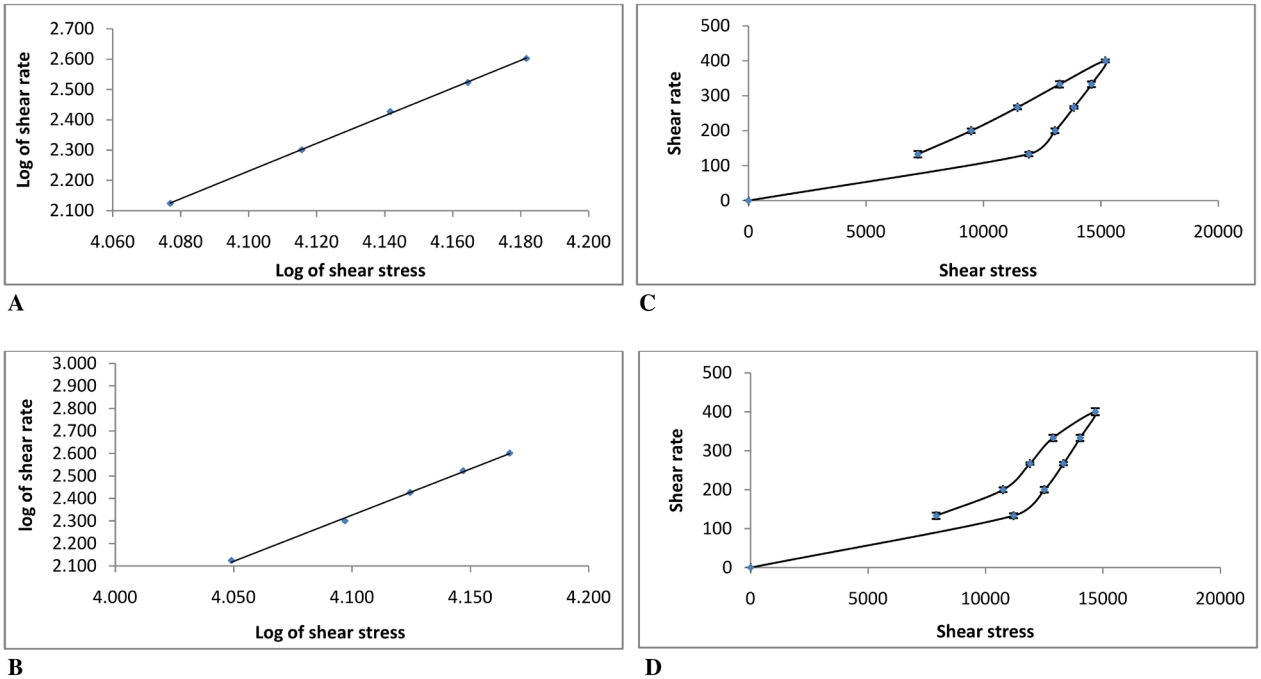
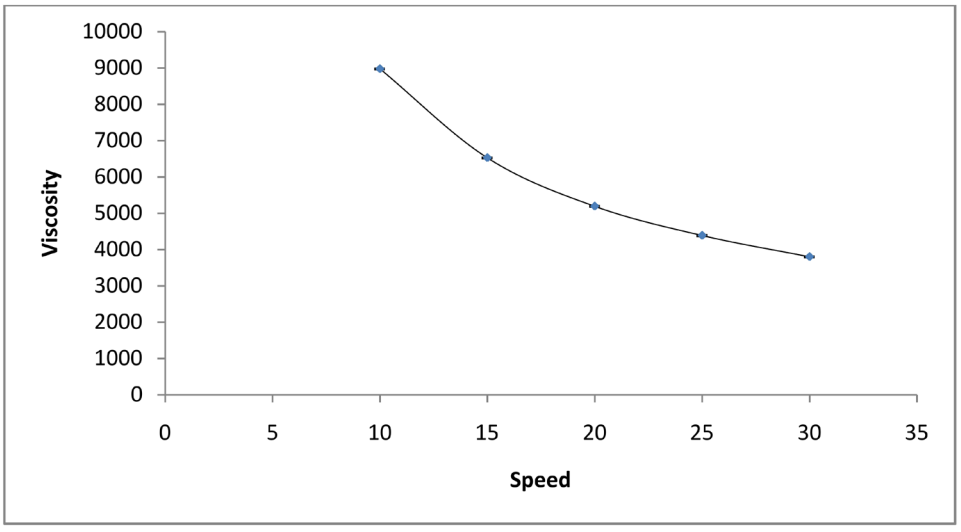


FIGURE 1 - Log shear stress vs. log shear rate plot of DSP_5 (A) and BSP_5 (B) Shear rate vs. shear stress plot of DSP_5 (C) and BSP_5 (D)



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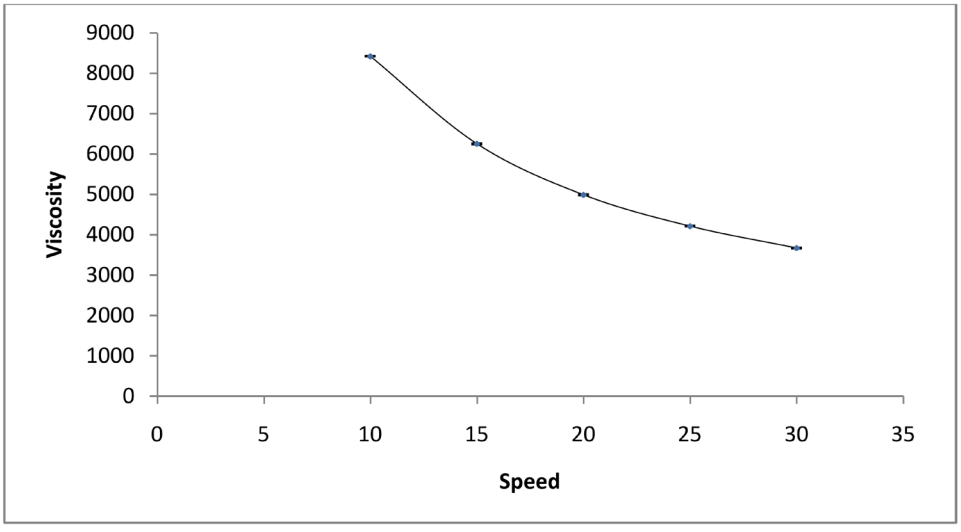


B

FIGURE 2 - Speed vs. viscosity plot of DSP_5 (A) and BSP_5 (B)

Spreadability

Good spreadability was one of the most optimal qualities a gel can possess. The therapeutic benefit of the gel also depends on the size of the region to which it readily spreads when applied. BSP gels were spreadable between 27 ± 2.0 mm and 63 ± 1 mm, while DSP gels were between 26 ± 2.0 and 60 ± 2.1 mm respectively (Table III). There was no statistically significant difference between the spreadability of BSP and DSP gels at $p > 0.05$

As gels increase in their viscosities, their spread diameter decreases. The spreadabilities of DSP_3 and BSP_3, prepared with 2.5 % HPMC, were 51 ± 0.06 and 55 ± 1.2, respectively higher than those of DSP_1 and BSP_1, prepared with 2.5 % Na CMC (37 ± 2.1 and 39 ± 3.0 respectively). However, DSP_5 and BSP_5 gels formed by blending of 1.25 % Na CMC and 1.25 % HPMC had the highest spreadability of 60± 2.1 and 63± 1.0 respectively (Shukr, Metwally, 2013).

Mucoadhesive strength

Mucoadhesion occurs due to hydration of bioadhesive polymer, where it results in a cohesive force as water is taken from the space between mucosa and polymer. BSP gels mucoadhesive strength ranged from 12.300 ± 0.004 g to 13.850 ± 0.003 g and DSP gels ranged between 12.600 ± 0.01 g and 13.900 ± 0.05 g respectively (Table III). No statistically significant difference was noticed between the mucoadhesive strengths of BSP and DSP gels.

An increase in the strength of mucoadhesion with an increase in polymer content was observed. The mucoadhesive strength of the DSP_2 (13.900 ± 0.05 g) and BSP_2 (13.850 ± 0.003 g) gels containing 3 % Na CMC was high, followed by DSP_4 (13.700 ± 0.005 g) and BSP_4 (13.400 ± 0.005 g) containing 3 % HPMC respectively. The formulations DSP_5 (12.600 ± 0.01 g) and BSP_5 (12.300 ± 0.004 g) containing 1.25 % Na CMC and 1.25 % HPMC showed least mucoadhesive strength (Sherafudeen, Vasantha, 2015). The gel formulations, DSP_5 and BSP_5, with the lowest viscosity, were highly spreadable (60 ± 2.1 mm and 63 ± 1 mm) among all formulations, yet had good extrudability and further showed good mucoadhesive strength.

pH

Gels pH should always match the pH of the region to which they are applied for treatment to minimize local irritation. The pH of BSP gels ranged from 6.4 ± 0.30 to 6.9 ± 0.10 and of DSP gels from 6.4 ± 0.20 to 6.7 ± 0.21 respectively (Table III). No statistically significant difference was noticed between the pH of BSP and DSP gels at $p > 0.05$.

The results showed that the pH of all prepared gels was approximately equivalent to the oral pH of saliva (pH 6.4), which suggested that the mucosa was not irritated. DSP_5 and BSP_5 gel formulations showed pH of 6.4 ± 2 and 6.4 ± 03 respectively (Hanan, Lena, Saba, 2018).

Drug Content Uniformity:

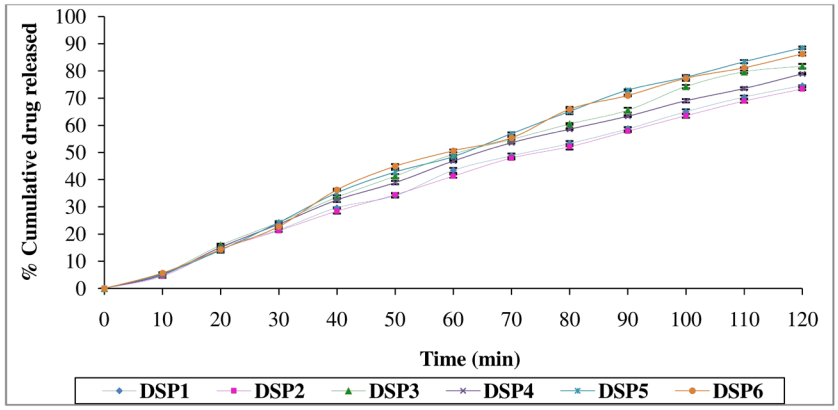
The percentage of drug content in BSP gel formulations ranged from 98.53 ± 0.185 to 99.94 ± 0.211 and the percentage of DSP gels ranged from 97.00 ± 0.599 to 98.80 ± 0.623 respectively (Table III) within the official limits (100 ± 5 %) suggesting that the drug was distributed evenly in the gel. There was no statistical significant difference between BSP and DSP gels in drug content at $p > 0.05$ (Pandit *et al*., 2007).

***In vitro* drug diffusion studies**

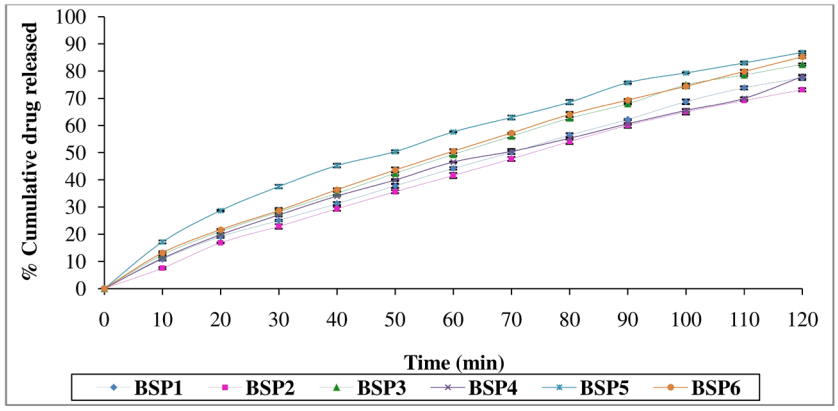
The DSP gel formulations showed cumulative drug release percentages ranging from 73.353 ± 0.753 to 88.473 ± 0.457 (Figure 3A). Cumulative release rates ranging from 73.353 ± 0.753 to 88.473 ± 0.457 % were seen in the DSP gel formulations (Figure 3A). The order of decreasing percentage of drug release in 2 h were BSP_5(86.869 ± 0.380) > BSP_6(85.228 ± 0.459) > BSP_3(82.432 ± 0.365) > BSP_4(77.994 ± 0.632) > BSP_1(77.508 ± 0.795) > BSP_2(73.070 ± 0.586) and DSP_5(88.473± 0.457) > DSP_6(86.228± 0.599) > DSP_3(81.737± 0.792) > DSP_4(78.892± 0.299) > DSP_1(74.551 ± 0.173) > DSP_2(73.353 ± 0.753) for BSP and DSP gels respectively.

For all gel formulations, regression coefficient values of zero, first order kinetic equations, Higuchi diffusion and Peppas log-log kinetics were almost 1, which means that plots were linear. The slope values of Peppas log-log plots were 0.883628 to 0.906036 and 0.94081 to 0.98480 respectively for BSP and DSP gels. For both the zero order and the first order plots, the regression coefficient values were determined and the results showed that the drug was released at zero order kinetics (Table IV). No statistically significant difference was noticed between the *in-vitro* drug diffusion of all gel formulations of BSP and DSP at $p > 0.05$ (Hanan, Lena, Saba, 2018). Due to their low viscosity, formulations DSP_5 and BSP_5 showed a higher *in-vitro* drug release of 88.473 60 ± 0.457 percent and 86.869 ± 0.380 percent compared to other formulations. Viscosity affects the drug release from the gel formulations. The increase in viscosity of the gels decreases *in-vitro* drug release observed in formulations BSP_2 (73.070 ± 0.586 %) and DSP_2 (73.353 ± 0.753 %).

The gel grows thicker and the water penetration decreases with increasing concentration of polymer with the decrease in the release of the drugs (Aslani, Zolfaghari, Fereidani, 2018).



A



B

FIGURE 3 - Cumulative percent drug released versus time plots (Zero order) Dexamethasone sodium phosphate (A) and Betamethasone sodium phosphate (B) from their gel formulations.

TABLE IV - Drug release kinetics of gel formulations

Formulation Code	Regression coefficient values				
	Zero-order plots (R^2)	First-order plots (R^2)	Higuchi Equation (R^2)	Korsmeyer-Peppas Equation (R^2)	Slope of Korsmeyer-Peppas Equation
DSP_1	0.996273	0.99311	0.97238	0.99356	0.94101
DSP_2	0.996799	0.99365	0.97207	0.99247	0.94081
DSP_3	0.99417	0.98911	0.97375	0.99457	0.96436
DSP_4	0.99348	0.99418	0.97535	0.99373	0.95573
DSP_5	0.99591	0.97956	0.96902	0.99416	0.98480
DSP_6	0.99265	0.98725	0.97114	0.99453	0.97843
BSP_1	0.996896	0.99023	0.978031	0.997639	0.892289
BSP_2	0.996401	0.995089	0.976499	0.999319	0.906036
BSP_3	0.994039	0.991233	0.982512	0.995815	0.903264
BSP_4	0.992372	0.989156	0.985089	0.995815	0.883628
BSP_5	0.980061	0.994062	0.995130	0.983851	0.892637
BSP_6	0.994194	0.986981	0.983491	0.994814	0.903216

Mechanical properties

Adhesiveness is an important parameter for the formulation of an oral gel, because it results in the successful delivery of therapeutic agents by promoting desired gel contact and gel retention at the target surface of the mucosa. The adhesiveness of the formulation was according to $BSP_2 > BSP_1 > BSP_4 > BSP_3 > BSP_6 > BSP_5$ and $DSP_2 > DSP_1 > DSP_3 > DSP_4 > DSP_5 > DSP_6$ for both BSP and DSP gels respectively (Table V).

As the concentration of the polymers was increased from 2.5 to 3 %, hardness as well as compressibility of the gels increased. BSP_2 and DSP_2 containing 3 % Na CMC showed maximum hardness (9.81 ± 0.02 N and 9.97 ± 0.03 N) and compressibility (39.85 ± 0.01 N mm and 40.02 ± 0.14 N mm) respectively, while BSP_5 and DSP_5 containing 1.25 % Na CMC and 1.25 % HPMC showed low hardness (5.75 ± 0.03 N and 6.13 ± 0.05 N) and compressibility (35.04 ± 0.02 N mm and 35.67 ± 0.02 N mm) respectively (Table V) (Jones, Woolfson, Djokic, 1996). As the polymer concentration increase from 2.5 to 3 percent, the cohesiveness of all the gels deteriorated. The BSP_5 and DSP_5 formulations had high cohesiveness (1.39 ± 0.04 and 1.32 ± 0.02), while the BSP_2 and DSP_2 formulations had low cohesiveness (0.93 ± 0.02 and 0.90 ± 0.03) respectively (Table V) (Jones, Woolfson, Brown, 1997).

Thus, among the twelve formulations, the BSP_5 and DSP_5 formulations exhibiting low hardness, low compressibility (facilitating easy removal from the container and application to the buccal mucosa), satisfactory adhesion and high cohesion (enabling the necessary gel adherence to the buccal mucosa and complete structural recovery of the gel after application) were considered to be the ideal gel formulations.

TABLE V- Mechanical properties of DSP and BSP containing oral mucoadhesive gels

Gel code	Hardness (N)	Compressibility (N mm)	Adhesiveness (N mm)	Cohesiveness
DSP_1	8.31±0.07	38.10±0.16	-37.95±0.06	0.98±0.01
DSP_2	9.97±0.06	40.02±0.14	-43.91±0.17	0.90±0.03
DSP_3	7.86±0.03	37.12±0.09	-35.90±0.12	1.10±0.01
DSP_4	9.48±0.02	39.33±0.01	-37.28±0.22	0.99±0.01
DSP_5	6.13±0.05	35.67±0.02	-34.07±0.11	1.32±0.02
DSP_6	7.28±0,02	36.70±0.06	-35.05±0.03	1.20±0.02
BSP_1	8.17±0.05	38.85±0.03	-37.02±0.04	1.00±0.03
BSP_2	9.81±0.04	39.85±0.01	-42.16±0.11	0.93±0.02
BSP_3	7.25±0.03	36.64±0.03	-35.87±0.12	1.18±0.01
BSP_4	9.13±0.02	39.00±0.11	-36.01±0.26	1.09±0.01
BSP_5	5.75±0.03	35.04±0.02	-33.99±0.10	1.39±0.04
BSP_6	6.76±0.09	36.11±0.10	-34.91±0.03	1.31±0.02

Drug polymer interaction studies

In order to characterize the potential interactions with the excipients used in preparing the formulations, the FTIR spectrums of betamethasone sodium phosphate and dexamethasone sodium phosphate were used. The wide band at 3272 cm^{-1} corresponds to the free hydroxyl groups within the dexamethasone sodium phosphate spectrum. The 1651 cm^{-1} peak was due to the functional group C=C. The peaks at 1031 cm^{-1} and 1209 cm^{-1} were due to functional groups of C-F and C-O-C respectively (Figures 4A and 4B) (Polshettiwar *et al*., 2019).

In betamethasone sodium phosphate spectrum, the 3417 cm^{-1} broad band corresponds to free hydroxyl groups. The peak was triggered by -OH stretching at 2941 cm^{-1}. The peaks at 1093 and 1047 cm^{-1} were due to the secondary hydroxyl group (characteristic peak –CHOH in cyclic alcohols, C-O stretch) and the primary –OH (characteristic peak $–CH_2$-OH in primary alcohol, C-O stretch) respectively. Asymmetrical and symmetrical stretching of carboxylate salt groups (Figures 5A and 5B) was assigned to the 1606 and 1454 cm^{-1} bands (Sneh*et al*., 2011). The FTIR spectrums of mucoadhesive gels that contain betamethasone sodium phosphate and dexamethasone sodium phosphate showed typical absorption bands almost equivalent to their respective pure drugs that did not indicate the interactions of the drug and excipient.

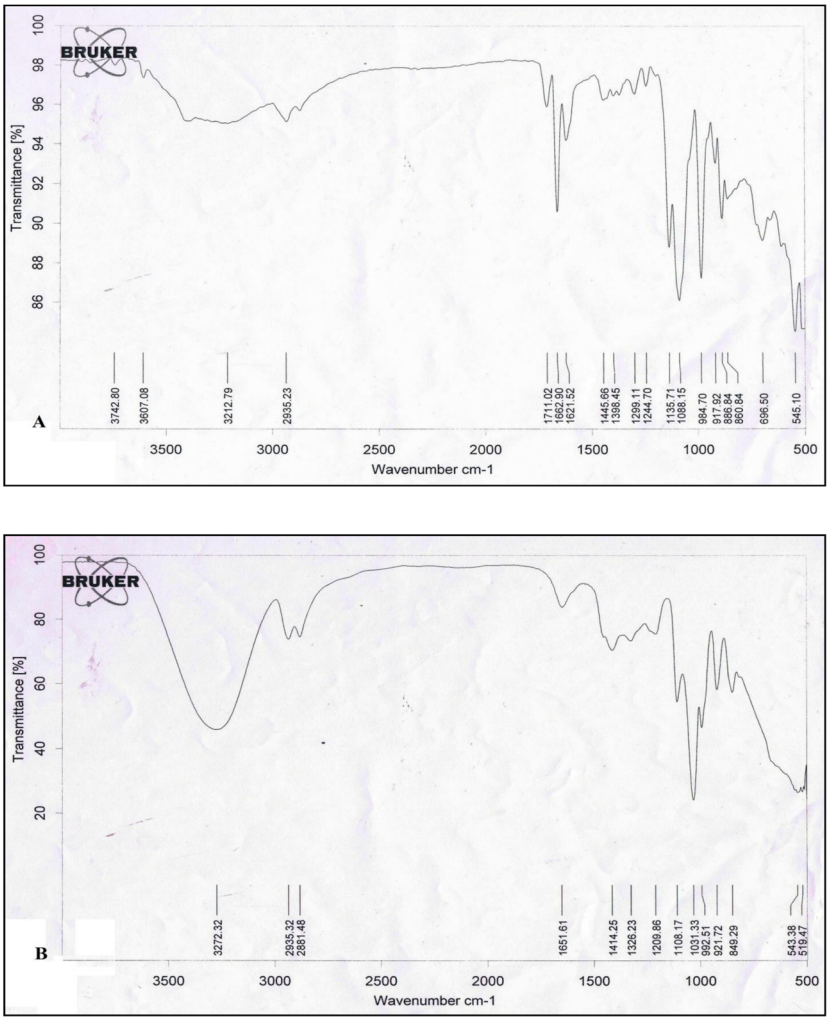


FIGURE 4 - FTIR Spectra of the Dexamethasone sodium phosphate alone (A) and with excipients and polymers (B) in the optimized formulation DSP_5

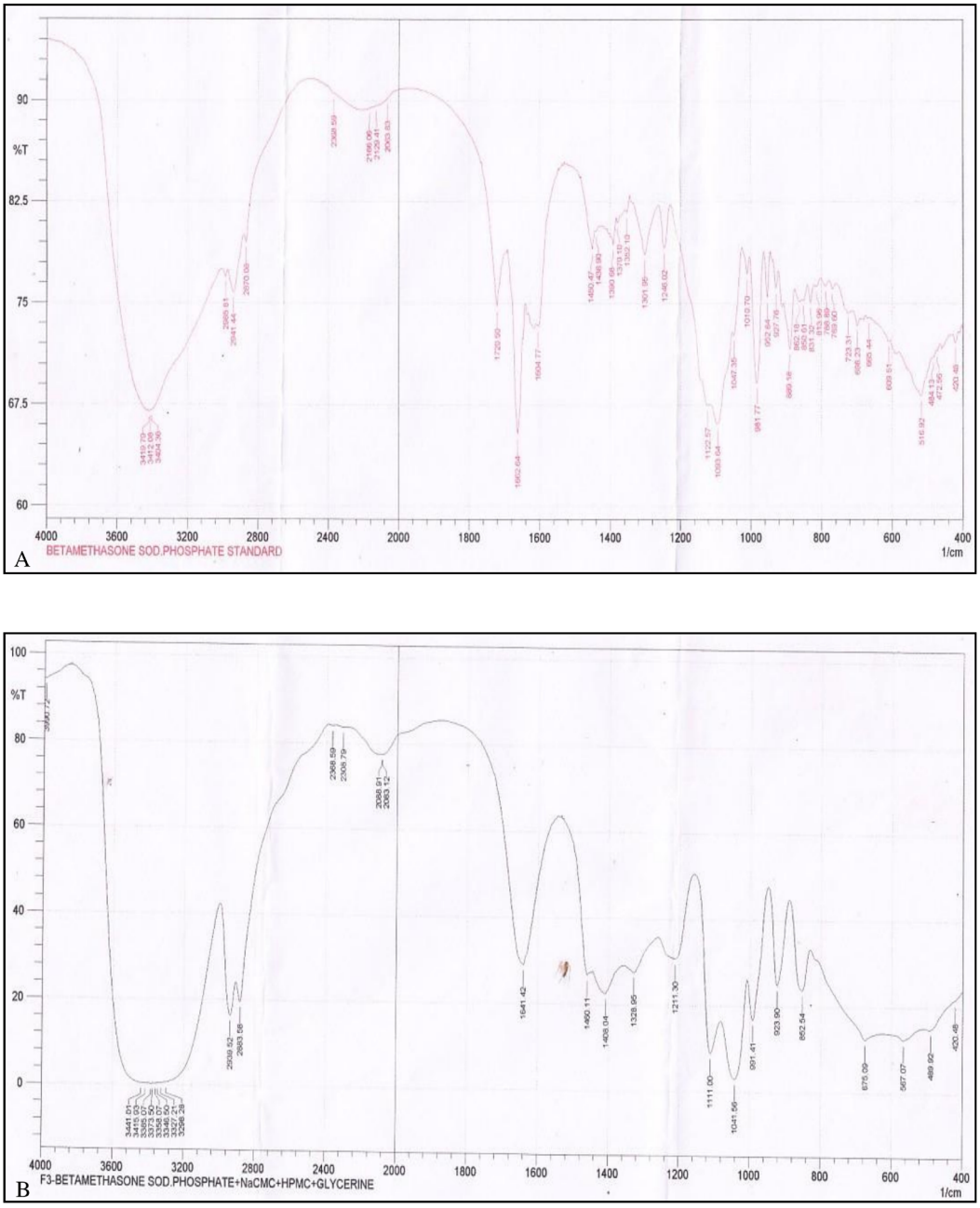


FIGURE 5 - FTIR Spectra of the Betamethasone sodium phosphate alone (A) and with excipients and polymers (B) in the optimized formulation BSP_5

Stability studies:

No physical changes were observed after 6 months of stability studies, such as color fading or the separation of liquid exudates from DSP_5 and BSP_5 formulations. During this period, the pH, drug contents of the gels were not influenced. There were no changes observed in extrudability, spreadability, mucoadhesive strength and other mechanical properties of the gels (Kumar, Verma, 2010).

In vivo studies

Precision formulations with good mucoadhesive strength and drug release properties were found to be BSP_5 and DSP_5 compared to other formulations. Hence these formulations were further subjected for *in-vivo* studies in OSMF induced rats.

The study focused on the range of histomorphological changes in oral mucosa following the local application of the BSP_5 and DSP_5 gel formulations, which were demonstrated by significant improvements in the mouth opening and body weight of OSMF induced rats ($p < 0.05$).

The histopathological evidence of OSMF induced rats under G-II, G-III and G-IV showed the redness of mucosa, visible epithelial lining and a very less amount of collagen in submucosa at the end of the first month of induction (Figure 6B, $p > 0.05$). Mucosa appeared light pink in the biopsy taken at the end of the second month, rare epithelial thickness and moderate elevation in the collagen content of submucosa were also observed (Figure 6C, $p < .05$). The results at the end of the 4th month showed remarkable changes such as white mucosal area development, invisible epithelial lining, and submucosal dense collagen formation (Figure 6D, $p < 0.01$). In a control group, however there was no change in oral mucosal redness, epithelial thickness and the content of collagen in the submucosa (Figure 6A, $p > 005$) (Kumar *et al*., 2007; Ali*et al*., 2014). Now in the treatment group with DSP_5 gel formulations (G-III), the atrophic epithelium, abundant collagen in submucosa, perivascular fibrosis, and chronic inflammation were shown at the end of the first month (Figure 7E, $p > 0.05$). At the end of the second month, biopsies showed a gradual renewal of epithelium to normal thickness and a moderate decrease in submucosal collagen (Figure 7F, $p > 0.05$). However, biopsies observed at the end of the 4th month showed radical differences in histopathological changes in oral mucosa with near to normal epithelium with 80 % collagen dissolution in submucosa (Figure 7G, $p < 0.01$).

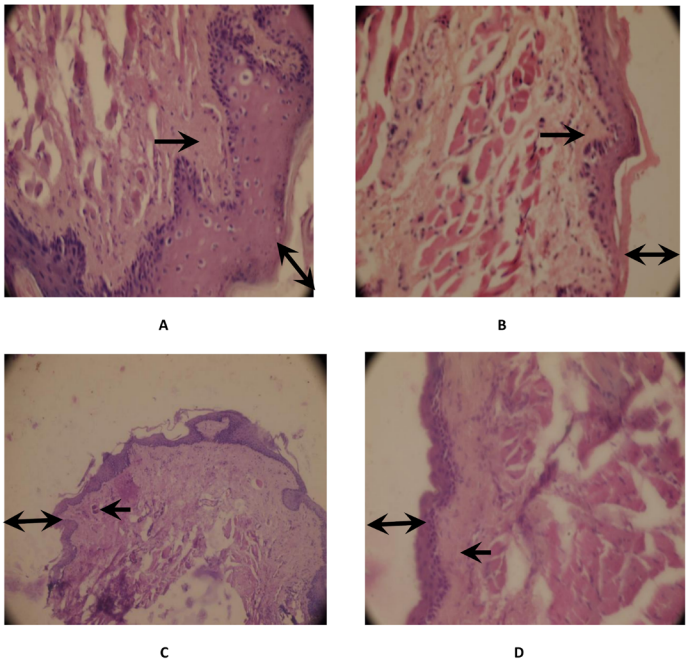


FIGURE 6 - Histopathological changes in the oral mucosa of rats during induction of OSMF at various observation periods (Haematoxylin eosin stain 10x magnification).

↔ Indicates thickness of epithelial lining
⇄ Indicates collagen content in submucosa

A-Normal buccal mucosa; B-Oral mucosa after 1 month induction showing no alterations in epithelial thickness, no significant change of collagen content in submucosa; C-Oral mucoca after 2 months induction showing a slight thinning of epithelial lining and moderately increased collagen content in submucosa; D-Oral mucosa after 4 months of induction showing a very thin epithelial lining, thick collagen deposition in submucosa and white patch in mucosa.

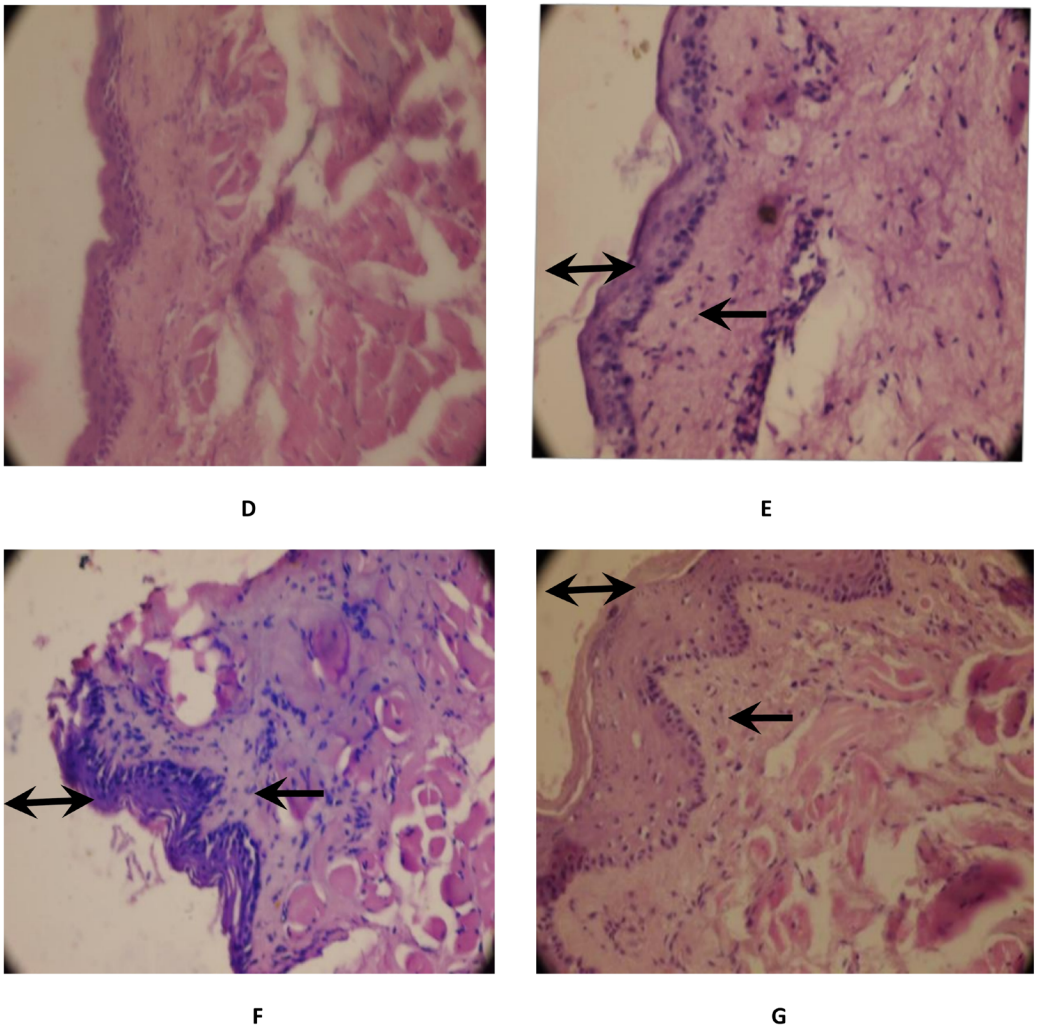


FIGURE 7 - Histopathological changes in the oral mucosa of rats during OSMF treatment with DSP_5 at various observation periods (Haematoxylin eosin stain 10x magnification).

↔ Indicates thickness of epithelial lining
↔ Indicates collagen content in submucosa

D--Oral mucosa after 4 months of induction showing a very thin epithelial lining, thick collagen deposition in submucosa and white patch in mucosa; E-Oral mucosa after 1 month treatment showing atrophic epithelium and dense collagen deposition in submucosa; F-Oral mucosa after 2 months treatment shows slight thickening of epithelial lining and moderate decrease in collagen content of submucosa G- Oral mucosal biopsy of 4th month showed the epithelial lining regaining its normal thickness and marked decrease in collagen content of submucosa.

While biopsies taken at the end of the 1st month showed a white patch in mucosa, atrophic epithelium and dense collagen deposition in submucosa, chronic inflammatory infiltrate, and perivascular fibrosis (Figure 8H, $p>0.05$) in the treatment group upon application of BSP_5 gel formulations (G-IV). At the end of the 2nd month, the biopsies showed pale mucosa, a very thin epithelial lining, less than 30 % collagen dissolution in the submucosa and inflammatory infiltrate presence (Figure 8I, $p > 0.05$). At the end of the 4th month, biopsies showed pale mucosa, thin epithelial lining, about 50 % collagen dissolution in submucosa and the presence of inflammatory infiltrates (Figure 8J, $p < 0.05$).

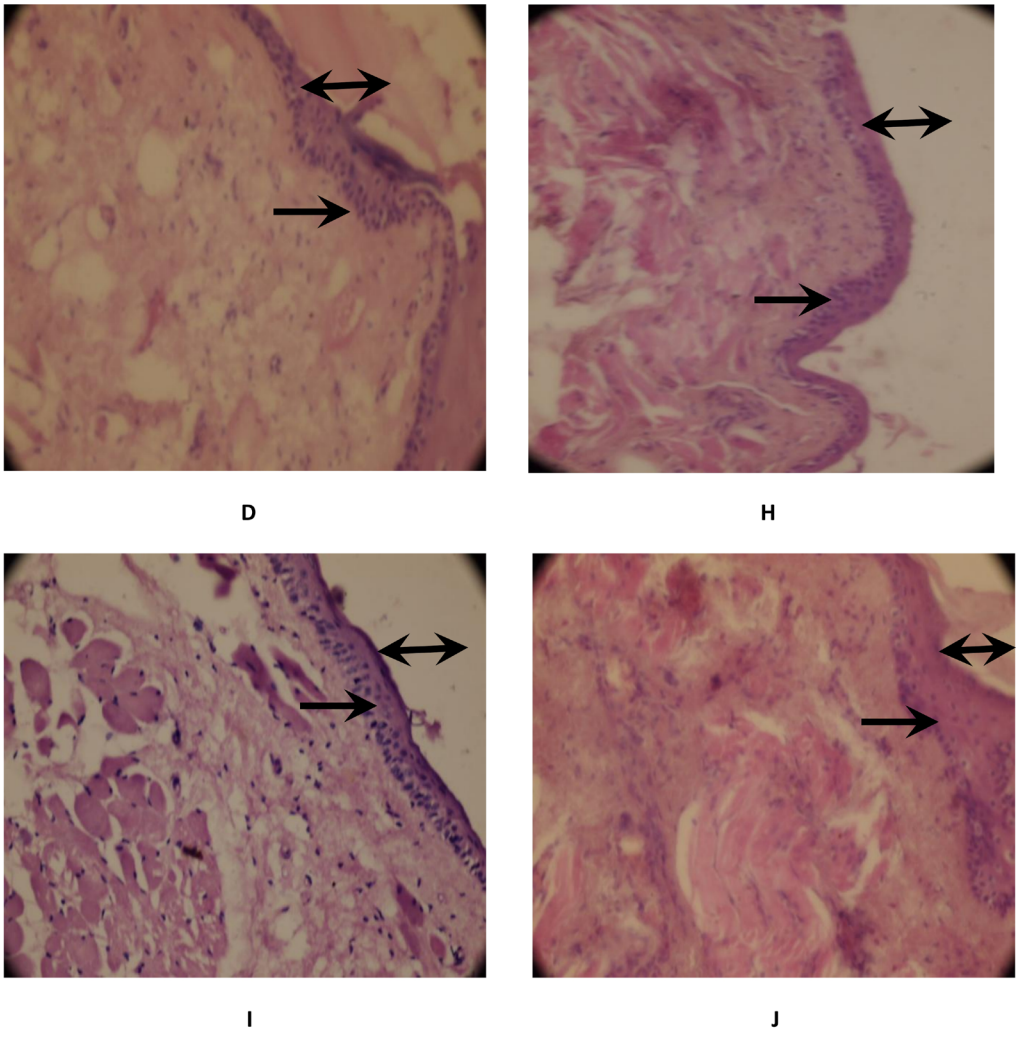


FIGURE 8 - Histopathological changes in the oral mucosa of rats during OSMF treatment with BSP_5 at various observation periods (Haematoxylin eosin stain 10x magnification).

Indicates thickness of epithelial lining
Indicates collagen content in submucosa
D--Oral mucosa after 4 months of induction showing a very thin epithelial lining, thick collagen deposition in submucosa and white patch in mucosa; H-Oral mucosa after 1 month treatment showing atrophic epithelium and dense collagen deposition in submucosa; I-Oral mucosa after 2 months treatment shows a very thin epithelial lining and decrease in collagen content of submucosa J- Oral mucosal biopsy of 4^{th} month showed thin epithelial lining and moderate decrease in collagen content of submucosa.

The epithelium had normal thickness and sub epithelial connective tissue was edematous with minimal collagen during the first month of OSMF induction. The epithelium was parakeratotic and a moderate increase of collagen tissue occurred in submucosa by the end of the second month induction. The epithelial filling was hyperplastic by the end of the fourth month, the loose conjunctive tissue and blood capillaries were replaced with dense deposits of collagen. OSMF induced rats had difficulty in opening their mouths and could not drink water from the feed bottle. Their activity, appetite, body weights were also reduced.

The G-III treated with DSP_5 demonstrated the highest dissolution in the second phase of the *in-vivo* study (treatment phase), which was evident by the epithelium retaining its normal thickness and re-appearance of loose connective tissue and blood capillaries in submucosa, whereas G-IV treated with BSP_5, still showed an atropic epithelia and only moderate dissolution of collagen in the end of 4th month experimental period. The 80 % improvement in DSP_5 compared to the 50 % improvement in BSP_5 was due to the corticosteroid mechanism preventing the action of inflammatory mediators released by sensitized lymphocytes and fibrosis prevention by decreasing fibroblastic proliferation and collagen deposition in the submucosa (Krishnamoorthy, Khan, 2013).

Astonishingly, body weight of the G-III and G-IV animals decreased from 145 ± 4 g to 92 ± 3 g and 146 ± 4 g to 91 ± 4 g for the first to fourth month of the induction of the OSMF respectively (Figure 9A, $p < 0.05$). In G-III and G-IV animals, a substantial weight increase ranged from 116 ± 4 g up to 185 ± 3 g (Figure 9B, $p < 0.05$) and from 90 ± 3 g up to 140 ± 2 g for 1 to 4 months following OSMF treatment (Figure 9C, $p < 005$) (Singh *et al*., 2012).

The above observations were clearly found in the G-III and G-IV treatment groups, suggesting that the mouth opening, consumption of food and weight of the treated animals were substantially increased. The DSP_5 formulation had a better and long-lasting effect. These results were supported by substantial increase *in-vitro* release of drug and enhanced antifibrotic properties of dexamethasone sodium phosphate in DSP_5, which warrant its prominent use as an OSMF mucoadhesive gel. There was no increase in mouth opening and body weight in G-II animals as they were left untreated after OSMF induction.

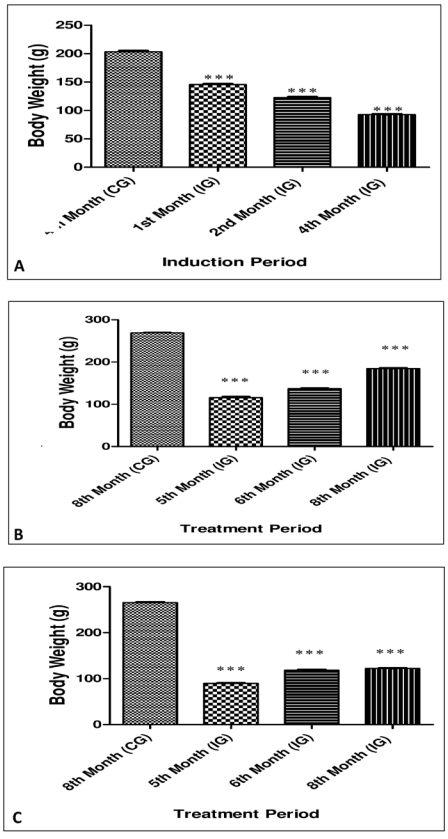
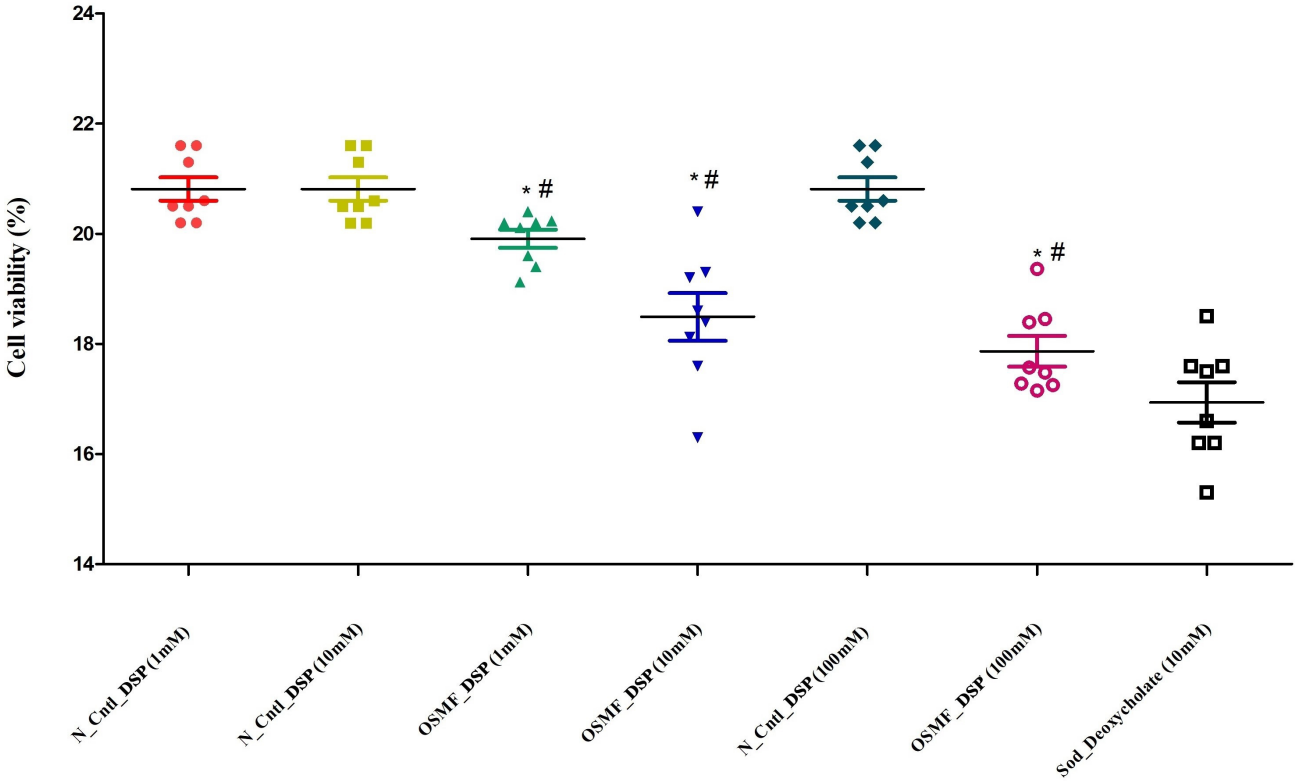


FIGURE 9 - Changes in the body weights of rats at different observation periods during OSMF induction (A) and OSMF treatment with DSP_5 (B) and BSP_5 (C); comparison between different groups were analyzed by One-way Analysis of Variance (ANOVA) using Dunnett multiple comparisons test by considering test vs control. *Indicates significance with respect to positive control group at $P < 0.05$.
CG = Control group, IG = OSMF induced group

Cytotoxicity study

MTT and LDH assays were used to determine *in vitro* cytotoxicity potential of various formulations. The cytotoxic effects of drug concentrations of 1 mM, 10 mM, and 100 mM in formulations were analyzed using MTT assay. As Fig. 10 reveals, dose-dependent cytotoxicity in OSMF cell lines treated at different concentrations compared to normal cell lines, where no change in cell viability was noticed. At 100 mM drug concentration, the highest cytotoxicity against OSMF cell lines was exhibited.



Data is expressed as Mean ± SD. * indicatates p< 0.01 when compared with control at 1mM, 10mM and 100mM respectively. # indicates that p < 0.05 when compared with Sodium Deoxycholate (10mM) for OSMF induced cells at DSP (1mM), DSP(10mM) and DSP(100mM) respectively.

FIGURE 10 - Cell viability of Normal and OSMF induced buccal cells following 24 h in-vitro exposure to Normal Cells (1mM, 10mM or 100mM) or DSP_5 (1mM, 10mM or 100mM) formulation or sodium deoxycholate.

The toxic effects of drug concentrations of 1 mM, 10 mM and 100 mM in formulations on membrane integrity of the cell lines were examined with the use of the LDH release assay. As seen in Table VI, the OSMF cell lines had more substantial LDH leakage at 100 mM drug concentration, whereas normal cell lines did not observe LDH leakage. The LDH activity, at 30 min in the incubation media of normal fibroblasts with drug formulations (1mM or 10mM or 100mM) was 13.12 ± 0.52; 12.87 ± 0.23; 13.23 ± 0.62 U/L/cm^2 respectively and was not significantly different from the LDH release at 120 min [(p = 0.654;0.351; 0.453)](Table VI).

Cytotoxicity reports showed that mitochondrial succinate dehydrogenase transforms MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) water soluble tetrazolium salt into formazan.

The insoluble formazan accumulates in metabolically active cells. Therefore, MTT is a measurement of mitochondrial activity in cells. The experimental results in this study did not report a cell death (Figure 10) at 1 mM, 10 mM, or 100 mM of DSP_5 gel formulation and did not harm the cellular viability of normal buccal cells, which indicates that the gel formulation was non-toxic to mucous epithelium. On the contrary, it was found that sodium deoxycholate had been shown to be cytotoxic at the concentration measured with major changes. The MTT cytototoxic test proved that DSP_5 was cytotoxic to cells induced by OSMF, which showed a decrease in cell viability, with an increment in gel formulation (dose-dependent) concentration and thus optimized gel-formulation was effective for treatments of OSMF. A substantial decrease ($p < 0.05$) of fibrosis in induced OSMF buccal cells was observed with 100 mM gel formulation (Berridge, Herst, Tan, 2005).

In addition to the MTT procedure, the membrane enzymes (LDH) of buccal mucosa were analyzed and compared between normal and OSMF induced buccal cells after the formulation DSP_5 (1 mM or 10 mM or 100 mM) and sodium deoxycholate treatment, as these biological markers were used for the assessment of mucus membrane toxicity. DSP_5 was shown to be harmless with no change in the leakage of LDH from normal buccal mucosal cells compared to sodium deoxycholate (Table VI) at the tested levels used in the analysis. However, there was a clear demarcation with increase in the LDH leakages over a period of 120 min upon treatment to OSMF cells indicating the gel formulation is cytotoxic to OSMF cells. In addition, it was also observed that no significant ($p > 0.05$) changes in LDH leakages were observed at different tested concentration of DSP_5 and thus require additional toxicity studies to be carried out (Kaja *et al.*, 2017; Surampalli, Nanjwade, Patil, 2015).

TABLE VI - The effect of DSF_5 (1mM, 10mM or 100mM) and Sodium deoxycholate on the release of LDH enzymes from the normal and OSMF induced buccal cells

DSF_5 (1mM) Formulation					
Enzyme activity	**Intestinal segment**	**30**	**60**	**90**	**120**
LDH (U/L/cm^2)	Normal buccal cells	13.12 ± 0.52*	14.51 ± 0.38*	13.24 ± 0.12*	14.19 ± 0.33*
	OSMF induced cells	21.45 ± 0.31*	22.36 ± 0.17*	23.17 ± 0.21*	27.11 ± 0.53*
Positive Control (Sodium deoxycholate, 10mM)					
Enzyme activity	**Intestinal segment**	**30**	**60**	**90**	**120**
LDH (U/L/cm^2)	Normal buccal cells	26.31 ± 0.51	29.23 ± 0.41	35.21 ± 0.23	38.21 ± 0.21
	OSMF induced cells	39.17 ± 0.23	41.25 ± 0.23	43.21 ± 0.31	48.16 ± 0.87
DSF_5 (10mM) Formulation					
Enzyme activity	**Intestinal segment**	**30**	**60**	**90**	**120**
LDH (U/L/cm^2)	Normal buccal cells	12.87 ± 0.23*	13.26 ± 0.41*	12.84 ± 0.31*	13.17 ± 0.31*
	OSMF induced cells	22.13 ± 0.34 *	23.03 ± 0.42 *	24.12 ± 0.23 *	26.12 ± 0.13*
DSF_5 (100mM) Formulation					
Enzyme activity	**Intestinal segment**	**30**	**60**	**90**	**120**
LDH (U/L/cm^2)	Normal buccal cells	13.23 ± 0.62 *	12.77 ± 0.38 *	13.26 ± 0.23*	14.56 ± 1.09 *
	OSMF induced cells	25.53 ± 0.45 *	27.25 ± 1.21*$^{\#}$	31.21 ± 1.03*$^{\#}$	35.67 ± 1.09*$^{\#}$

TABLE VI - The effect of DSF_5 (1mM, 10mM or 100mM) and Sodium deoxycholate on the release of LDH enzymes from the normal and OSMF induced buccal cells

		DSF_5 (1mM) Formulation			
Enzyme activity	**Intestinal segment**	**30**	**60**	**90**	**120**

Values are mean ± SD for 6 rats in each group. * $p < 0.01$ showing statistically significant when compared with the positive control (sodium deoxycholate). # indicates $p < 0.05$ showing statistically significant when compared with DSF_5 (1mM and 10mM) respectively.

CONCLUSION

While the latest advanced OSMF treatment using steroidal intralesional injections were deemed curative; multiple injuries, pain, overdose and patient non-compliance were associated with them. Therefore the present study results are novel and unique with non-invasive, improved, conclusive OSMF treatment obtained by the use of drugs such as DSP and BSP as mucoadhesive gels. The application of local gel decreases painful lesions and morbidities associated with invasive treatment approaches to a minimum. Therefore the formulation DSP_5 can be used as the cost-effective, self-applicable and patient compliance gel for OSMF management. In addition, there were no modifications of the cell viability and membrane markers of normal buccal mucosal cells in assays for leakage of MTT and LDH that claimed that the prepared gel formulation was cytotoxic to OSMF buccal cells and not harmful to normal cells. Therefore, for the successful treatment of OSMF patients, the DSP_5 may be a promising gel formulation. However, more *in vivo* toxicity studies and clinical trials are required for their thorough assessment.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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