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FORMULATION AND EVALUATION OF SUCRALFATE PASTE FOR CHEMOTHERAPY INDUCED ORAL MUCOSITIS

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ABSTRACT

Oral mucositis is the most common acute side effect experienced by patients undergoing chemotherapy. The present study was aimed at developing sucralfate paste by polymerization method to treat oral mucositis using mucoadhesive polymer for site specific and controlled action in oral mucosa. The formulated formulations were characterized in terms of drug content, viscosity, pH, tube extrudability, spreadability, stability and *in vivo* evaluation. Oral mucositis was induced in male wistar rats by 5 - Fluorouracil and glacial acetic acid and then the treatment was given by applying the sucralfate paste. All the formulations passed all the above tests. The SP 8 formulation which had higher viscosity, spreadability and tube extrudability was chosen as the best formulation and evaluated for its stability and *in vivo* efficacy. Assessment of oral mucositis indicates that the SP 8 formulation is safe and more effective in healing oral mucositis with statistically significant ($P<0.05$).

KEYWORDS: Sucralfate, oral mucositis, mucositis score, polymerization.

INTRODUCTION

Cancer is a major public health problem worldwide and is the second leading cause of death globally.[1] One of the mostly followed methods of treatment for the cancer is chemotherapy. There are many oral complications related to the treatment and the most prevalent are oral mucositis. This is accompanied by pain, burning and discomfort, which are greatly aggravated by contact with highly spicy foods. Clinically, mucositis is characterized by inflammation, erythema, mucosal atrophy, exacerbation and ulceration of the oral mucosa

with or without pseudo membrane.[2] Severe symptoms can interrupt the delivery of treatment.[3]

Sucralfate is a well-known drug used for ulcers and comes under the category of mucosal protectives. It is a locally acting drug with 5% bioavailability. In the acidic pH, sucralfate polymerizes into a paste, with its strong negative charge, it binds to exposed positively-charged proteins at the base of ulcers crater and forms a physical barrier that protects the ulcer surface from further injury. Sucralfate may increase prostaglandin production. Prostaglandins are known to protect the mucosal lining and may also believe to bind epithelial growth factor and fibroblast growth factor, both of which enhance the growth and repair mechanism of the mucous.[4,5] When it is applied to the affected area, site specific action takes place and so the efficacy will be increased. Hence various formulations of sucralfate paste were prepared by polymerization method using chelating agent, crosslinking agent, mucoadhesive polymer, viscosity increasing agent, emollient, sweetening agent, preservative etc. The optimized formulation was evaluated for its efficacy by animal study in male wistar rats.

MATERIALS AND METHODS

Materials

Sucralfate was obtained from Zhejiang Haisen Pharmaceutical Co. Ltd, China. Citric acid was procured from Canton Laboratories, Gujarat. Calcium carbonate was procured from Gangotri Inorganic Pvt Ltd, Gujarat. Magnesium sulphate was procured from Canton Pharmaceuticals, Gujarat. Xanthan gum was procured from Rhodia Chemie, Europe. Hydroxyl ethyl cellulose was procured from Hercules. Glycerin was procured from Godrej Industries, Mumbai. Sorbitol was procured from Gulshan Polyols Ltd, India. PEG 400 was procured from Laffans Petro (Huntsman), Gujarat. Propylene glycol was procured from Manali Petrochemicals, Chennai. Liquid paraffin was procured from Savita Chemicals, Mumbai. Titanium dioxide was procured from Kronas, Mumbai. Silica (ppt) and Silica (absil) was procured from Madhu Silica Pvt. Ltd, Gujarat. Saccharin sodium was procured from Tianjin Changjie Chemicals Co. Ltd, China. Sodium benzoate was procured from Navyug Pharmachem Pvt. Ltd., Gujarat. All chemicals used were of analytical grade. Animal experiments were approved by the Institutional Animal Ethical Committee under Reg. No.321/PO/Re/S/01/CPCSEA dated: 30/08/2018.

Methods

Drug - excipient interaction study

Drug - excipient interaction study was carried out to determine the possible interaction between drug and excipient (citric acid). Differential scanning calorimetry (DSC) was performed by using differential scanning calorimeter (TA, Model Q200) equipped with computer analyzer. About 5 mg of the sample was placed in an aluminum pan and scanned in the temperature over 0-500° at a heating rate of 10°/ min under a nitrogen purge at 50 ml/min.[6,7] Fourier transform infra-red spectroscopy (FTIR) was carried out using Bruker FT-IR spectrophotometer. Potassium bromide (KBr) pellet method was employed. The disc was placed in the spectrophotometer and the spectrum was recorded in the range of 4000 to 500 cm^{-1}.[8]

Formulation of sucralfate paste

The sucralfate paste was prepared by using planetary mixer. The composition is given in Table 1. Sodium benzoate and saccharin sodium were accurately weighed and dissolved in distilled water. Glycerin, sorbitol, PEG 400 and propylene glycol were added and mixed for 5 min at 50 rpm. Citric acid was accurately weighed and dissolved separately in sufficient amount of distilled water and this solution was added to a mixture of sucralfate, calcium carbonate and magnesium sulfate and mixed for 5 min to form a paste separately. This paste was added to the above mixture and mixed for 10 min in planetary mixer. Then silica (Precipitated), silica (Absil), titanium dioxide and xanthan gum were accurately weighed and sifted through sieve No. 20, mixed for 5 min and slowly added to the contents of the planetary mixer under mixing at 50 rpm. The contents of the paste which adhered to the blades and the sides of the planetary mixer was removed, liquid paraffin was added and allowed to mix for 1 h at 50 rpm. Then it was weighed, filled in tubes and labeled.

Table 1: Formulation of sucralfate paste.

Ingredients (g)	SP 1	SP 2	SP 3	SP 4	SP 5	SP 6	SP 7	SP 8
Sucralfate	10	10	10	10	10	10	10	10
Citric acid	1	1	1	1	1	1	1	1
Calcium carbonate	1.25	0.5	0.6	0.6	0.5	0.5	0.5	0.5
Magnesium sulfate	1.25	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Xanthan gum	1	-	1	1	0.55	1.15	0.6	0.4
Hydroxy Ethyl Cellulose	1	1	-	0.1	0.08	0.08	-	-
Glycerin	35	-	20	10	10	10	30.3	23
Sorbitol	-	-	-	20	30	20	25	20

PEG 400	-	-	-	11	3	11	-	-
Propylene glycol	-	23	-	-	-	-	5	5
Liquid paraffin	-	-	-	-	-	-	-	2
Silica (ppt)	-	-	-	10	7	3	10	8
Silica (absil)	-	-	-	-	-	-	6.6	5
Titanium dioxide	-	-	-	0.2	0.15	0.15	-	-
Sodium benzoate	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Saccharin sodium	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Water	49.1	63.6	66.5	35.2	36.82	42.22	10.1	24.2

Evaluation of sucralfate paste

Content of sucralfate estimated as content of Aluminum

One gram of sample was accurately weighed in a 250 ml conical flask, 10 ml of 6 N hydrochloric acid was added, mixed and heated in a water-bath with continuous stirring at 70° for 5 min. It was cooled to room temperature, diluted with water and filtered. To 25 ml of the filtrate, 25 ml of 0.05 M EDTA and 20 ml of acetic acid - ammonium acetate buffer was added and again heated in a water-bath at 70° for 5 min, then cooled. 50 ml of alcohol, 2 ml of dithizone was added and titrated with 0.05 M zinc sulfate until the colour changes to bright rose pink. Blank was estimated. Each ml of 0.05 M EDTA is equivalent to 0.001349 g of Aluminum.[9]

Estimation of viscosity

Rheological measurements can be regarded as sensitive tools for detecting structural changes in pharmaceutical paste and should be regarded as an integral part of the quality evaluation of pharmaceutical paste. Viscosity of the paste was determined by using Brookfield viscometer at 25°. Helipath spindle S 96 attached to viscometer was immersed in the jar filled with paste and the viscosities were measured by rotating the spindle at 1 rpm.[10]

Determination of pH

One gram of the paste was accurately weighed and dispersed in 10 ml of purified water and pH was determined by using pH meter (Digisun Electronics).[11,12]

Tube extrudability

The method adopted for evaluating extrudability was based upon the quantity in percentage of paste extruded from tube on application of certain load. More the quantity extruded better was its extrudability. The formulations were filled into a clean, lacquered aluminum collapsible tube with a 5 mm opening. It was then placed in between two glass slides and was clamped. Extrudability was determined by weighing the amount of paste extruded through

the tip when a constant load of 1 kg was placed on the slides and paste extruded was collected and weighed. The percentage of paste extruded was calculated. The comparative extrudability of the formulations were noted.[13]

Spreadability

Spreadability is a term expressed to denote the extent of area to which the topical application spreads on application to skin on the affected parts. The therapeutic efficiency of the formulation also depends upon its spreading value. Hence, determination of spreadability is very important in evaluating topical application characteristics. For the determination of spreadability, excess of paste (3g) was applied in between two glass plates and was compressed to uniform thickness by placing 1000 g weight for 5 minutes. Thereafter weight (50g) was added to the pan and the top plate was subjected to pull with the help of string attached to the hook. The time in which the upper glass slide moves the lower plate to cover a distance of 10 cm is noted. A shorter interval indicates better spreadability. The spreadability (S) was calculated using the formula S = m.l/t where, S is spreadability, m is weight tied to upper glass slide, l is length moved on glass slide and t is time.[14]

Stability studies

Stability of the paste was determined by storing the sample filled in the sterile lacquered aluminum collapsible tubes at the accelerated stability condition of 40°±2°, 75% RH±5% RH and parameters like colour, odour, taste, pH, viscosity and drug content was evaluated for a period of three months.[15]

***In vivo* evaluation**

Eighteen Male Wistar rats, weighing 250-300g were randomly divided into three groups of 6 animals each and tagged as Group 1 (Control), Group 2 (Induction of oral mucositis) and Group 3 (Induction of oral mucositis and treatment). Animals were housed at 25±2° under a 12/12 h light-dark cycle with access to feed and water *ad libitum* and weighed at the beginning and at the end of the experiment.

Chemotherapy induced oral Mucositis and Treatment

Oral mucositis was induced by administration of the 5-Fluorouracil (30 mg/kg, i.p.) on 0^{th}, 5^{th} and 10^{th} day to Group 2 and 3. On day two, the animals were anesthetized using [ketamine hydrochloride 10% (50 mg/kg) and xylazine hydrochloride 2% (5 mg/kg i.p.), 0.4 ml per animal] and right cheek mucous was chemically injured by applying swab soaked in 10 µl

solution of glacial acetic acid 96% (50% v/v) in distilled water for 60 s as shown in Fig. 1. The treatment was given only to group 3 from 12th to 18th day. Polymerized sucralfate paste was applied twice a day to mucositis using disposable flexible swabs and the animals were prevented from drinking water or eating for 30 min.

Fig. 1: Induction of oral mucositis by 50% glacial acetic acid.

Assessment of oral mucositis

Oral mucositis was observed in all the groups on days 5, 10, 12, 14, 16 and 18. The mucositis was scored according to the mucositis scoring system as shown in Table 2 and it is statistically analyzed by ANOVA. To evaluate the histopathological changes in the oral mucosa, animals from each group were sacrificed by deep anaesthesia using ketamine hydrochloride [10% (100 mg/kg) and xylazine hydrochloride 2% (10 mg/kg) i.p., 0.5ml per animal] on 15th and 18th day. Right cheek mucous was removed from each animal as shown in Fig. 2, placed in 10% neutral buffered formalin solution, dehydrated, diaphonized, embedded in paraffin, sectioned at a thickness of 5 µm, stained with hematoxylin-eosin (HE) and examined under microscopy.[16,17]

Table 2: Mucositis score system.

Scores	Description
0	Normal
0.5	Slightly pink
1	Slightly red
2	Severe reddening
3	Focal desquamation

4	Exudation covering less than one-half of lip
5	Exudation covering more than one-half of lip

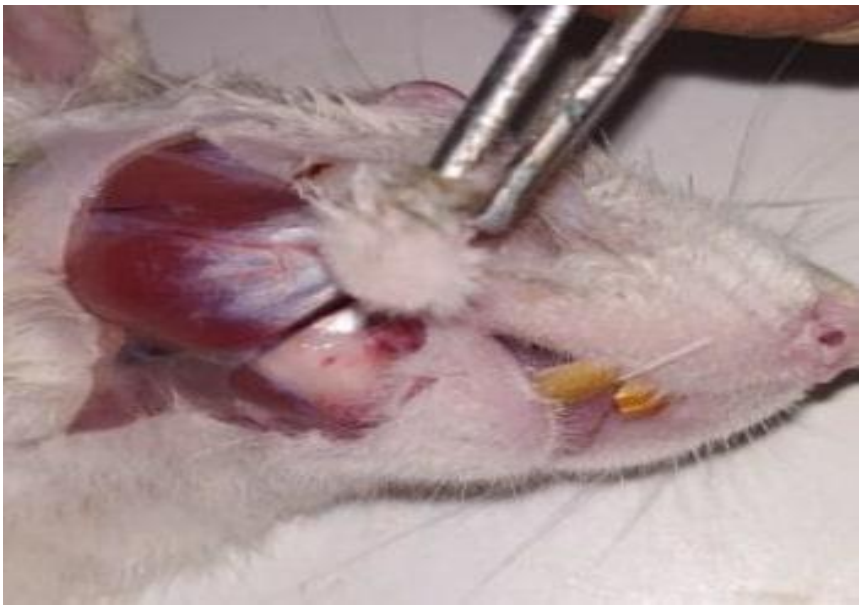

Fig. 2: Removal of oral mucosa.

RESULTS AND DISCUSSION

Differential scanning calorimetry (DSC) thermogram of sucralfate, citric acid and the mixture of sucralfate and citric acid as a polymerized paste is shown in Fig. 3-5 respectively. Sucralfate thermogram shows a broad endothermic peak, this reveals that the sucralfate melts at 140.43°. DSC thermogram of citric acid revealed a sharp endothermic peak at 155.75° which is its melting point and another broad endothermic peak shows the decomposition at 220.49°. And the first small endothermic peak may be due evaporation of water molecules. From the shift of the peak of sample from their original position, as compared to sucralfate and citric acid, it is confirmed that there is an interaction between sucralfate and citric acid.[18,19]

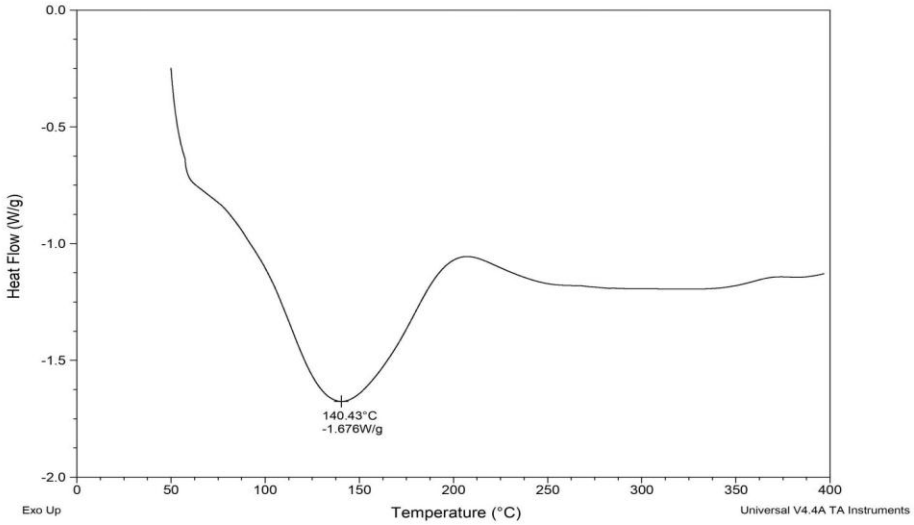

Fig. 3: DSC thermogram of sucralfate.

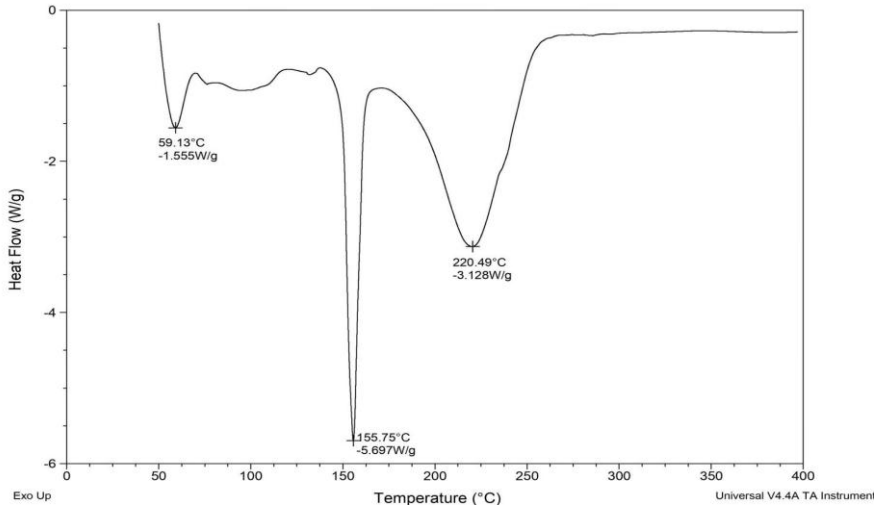

Fig. 4: DSC thermogram of citric acid.

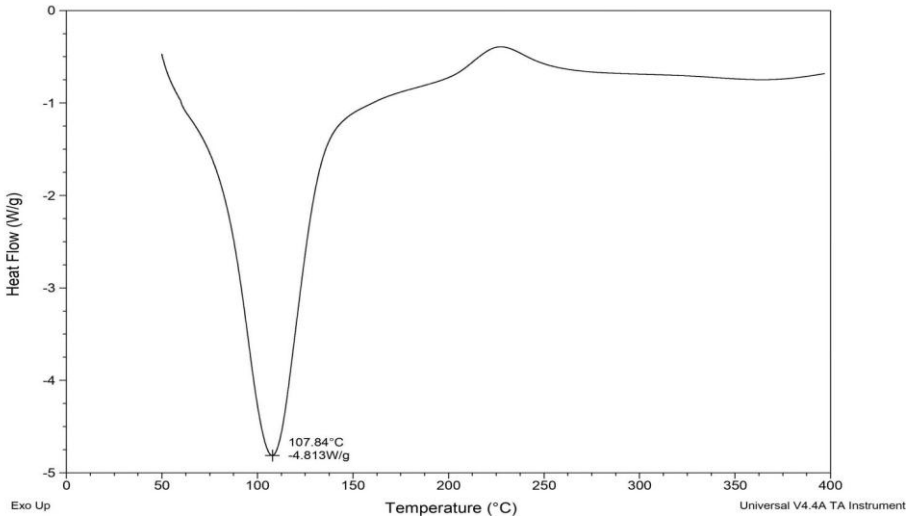

Fig. 5: DSC thermogram of mixture (Sucralfate and citric acid).

The FTIR spectrum of sucralfate and the mixture of sucralfate and citric acid as a polymerized paste is shown in Fig. 6-7 respectively. Hetero-aromatics such as furan, pyran shows C-H stretching bands in the region 3077-3000 cm^{-1}. So, the peak at 3048.55 cm^{-1} in drug and the peak at 3033.51 cm^{-1} in mixture indicates C-H stretching of hetero-aromatics. The broad band characteristic for hydroxide group is observed in the range of 4000-2500 cm^{-1} and 1800-1300 cm^{-1} and it is present in both the sample. The peaks obtained in drug in the region of 1800-1300 cm^{-1} are 1730.18 cm^{-1}, 1633.19 cm^{-1}, 1519.06 cm^{-1} and 1366.56 cm^{-1} and in paste only two peaks were observed at 1732.16 cm^{-1} and 1489.13 cm^{-1} which may be due the chemical interaction between the drug and the citric acid.[20]

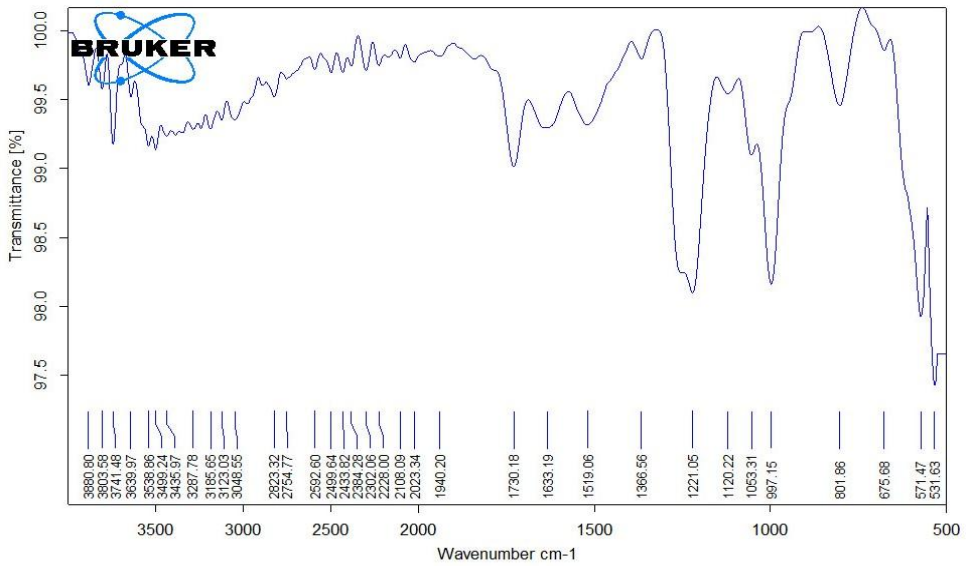

Fig. 6: FTIR spectrum of sucralfate.

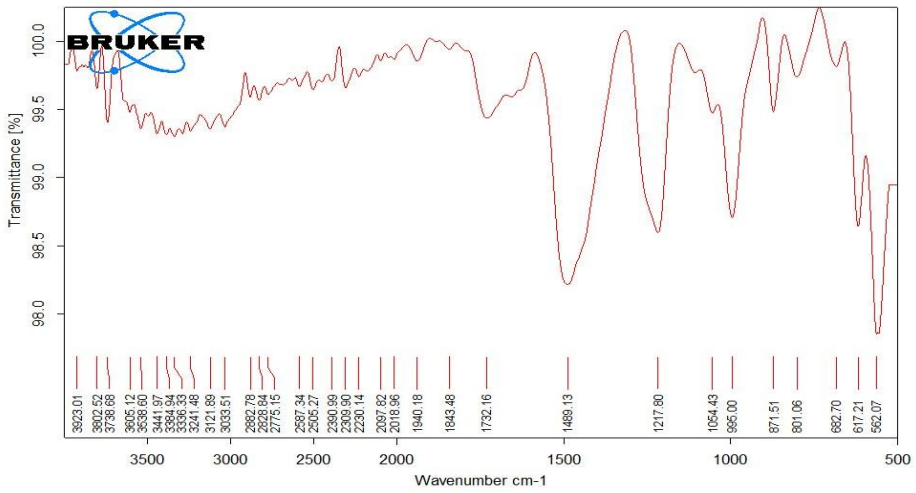

Fig. 7: FTIR spectrum of mixture (Sucralfate and Citric acid).

Sucralfate paste was evaluated and is shown in Table 3. The content of sucralfate estimated as content of aluminum was carried out by titration method and the percentage of aluminum present in all the formulations is in the limits of 15.5 to 18.5% of Aluminum, complies with the official USP pharmacopoeia. The pH of all the formulations was found to be in the range of 6.20 - 7.07 and so these pastes can be considered non-irritant to the buccal cavity. The viscosity, tube extrudability and spreadability of the formulation SP 8 was found to be good when compared to the other formulations and so this was considered as a finalized formulation.

Table 3: Physicochemical evaluation of sucralfate paste.

Formulation code	Drug content (% Al)	Viscosity (cp)	pH	Tube Extrudability (%)	Spreadability (g.cm/sec)
SP 1	15.82±0.06	329651.92±0.7	7.07±0.05	69.25±0.49	4.04±0.31
SP 2	16.63±0.02	287354.75±0.4	6.73±0.03	67.35±0.34	3.58±0.09
SP 3	17.31±0.03	319863.43±0.5	6.20±0.06	68.60±0.56	3.45±0.57
SP 4	18.12±0.02	507952.84±0.9	6.35±0.04	86.90±0.37	5.16±0.45
SP 5	16.50±0.03	462589.05±0.8	6.94±0.02	89.35±0.35	5.33±0.57
SP 6	18.41±0.01	509972.82±0.4	6.82±0.01	92.05±0.28	5.58±0.26
SP 7	17.04±0.03	479843.43±0.9	6.63±0.03	89.75±0.34	5.50±0.12
SP 8	17.85±0.05	548649.20±0.3	7.01±0.02	93.35±0.25	5.79±0.05

Mean±SEM was calculated from three observations

A stability study was carried out for optimized formulation SP 8 and is shown in Table 4. The parameters like colour, odour, taste, pH, viscosity and drug content were evaluated and compared to the freshly prepared formulation and observed that there was no change in the physical and chemical properties of the paste. It indicates that the formulation is stable.

Table 4: Stability studies.

Parameters	40±2 °, 75% RH±5% RH			
	Initial	1st month	2nd month	3rd month
Colour	White	White	White	White
Odour	No odour	No odour	No odour	No odour
Taste	Sweet	Sweet	Sweet	Sweet
pH	7.41±0.02	7.37±0.05	7.35±0.03	7.33±0.05
Viscosity (cp)	548649.20±0.3	548345.31±0.8	547963.64±0.6	547956±0.5
Drug content (% Al)	17.85±0.05	17.83±0.02	17.75±0.05	16.59±0.03

The animal study was carried out in Male wistar rats for a period of 18 days to estimate the effectiveness of the formulation SP 8 in curing the oral mucositis. The oral mucositis formed

in the Group 2 and Group 3 rats was visually observed as shown in Fig. 8-11 and scored according to the severity of the condition. The results indicated that the progression of oral mucositis was increased day by day. In Group 2 the weight of the animals was found reduced due to the inability of the animal to eat or drink water. The severity of the oral mucositis was found to decrease from 13th day to 18th day in Group 3 animals due to the treatment with sucralfate paste. Animal weight in Group 3 was reduced slightly till 10th day and after the treatment was started further decrease was not observed. The Mean±SEM are calculated and it was statistically analyzed by ANOVA is graphically represented in Fig. 12 the P value found to be 0.0069; it is statistically significant ($P < 0.05$).

Fig. 8: Slightly pink oral mucositis at 2nd day in Group 2.

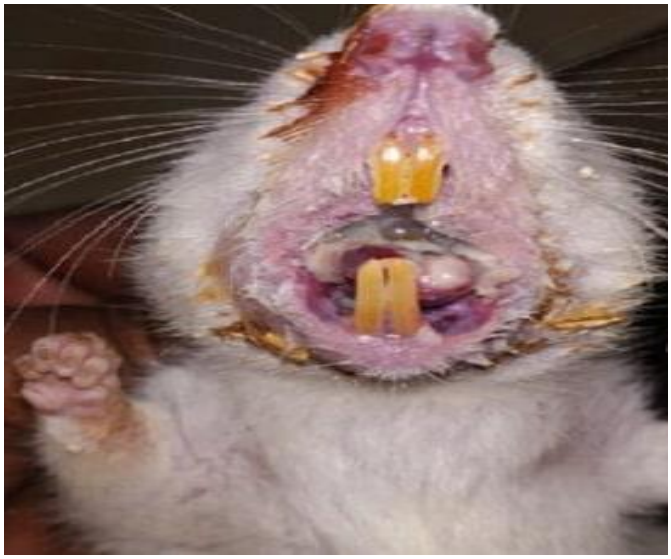

Fig. 9: Complete ulceration of the oral cavity at 10th day in Group 2.

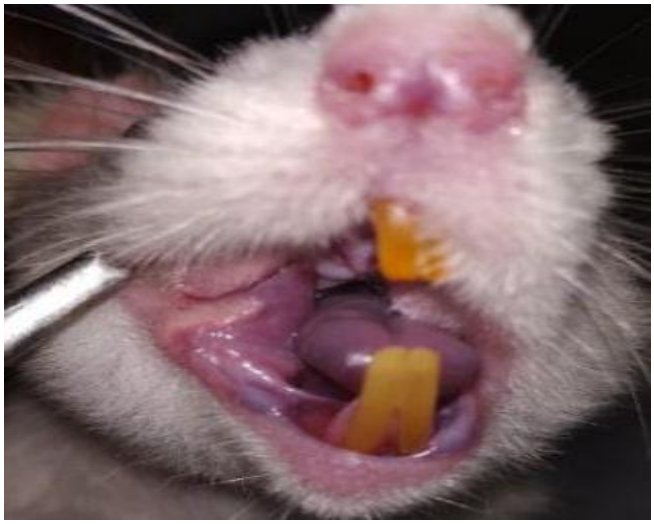

Fig. 10: Incomplete healing at 15th day in Group 3.

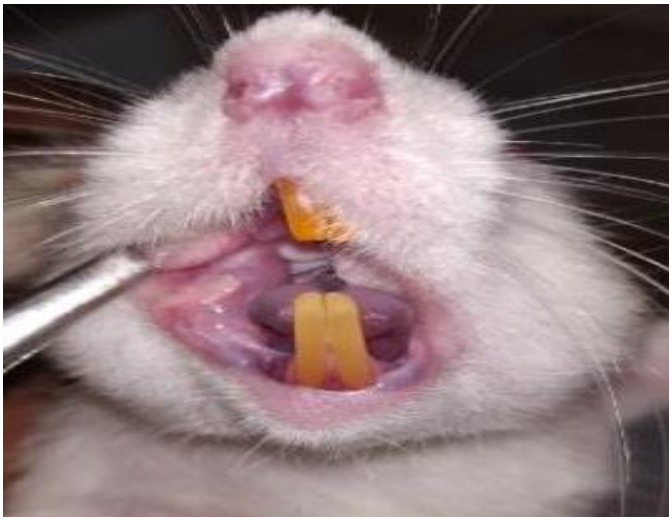

Fig. 11: Complete healing of oral mucositis at 18th day in Group 3.

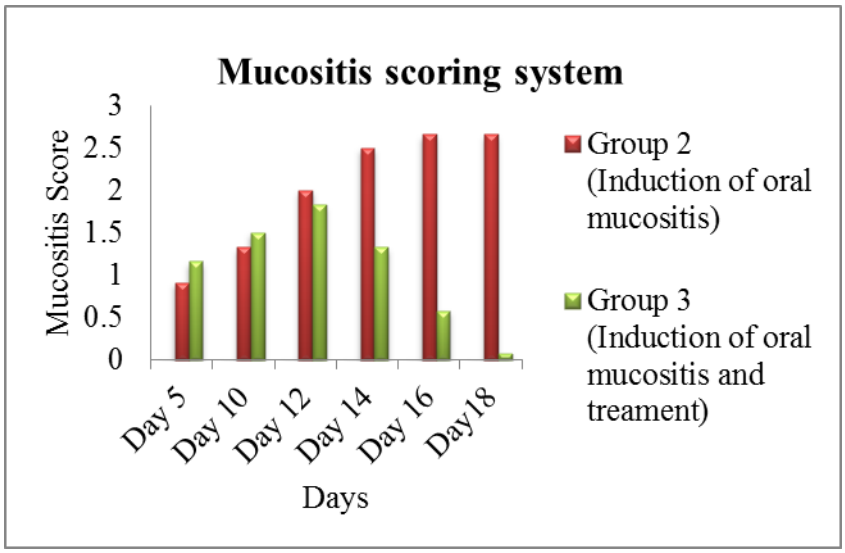

Fig. 12: Mucositis scoring system.

Histopathological sections were observed and are shown in Fig. 13-17. As the Group 1(control) had no intervention, it represents the normal tissue lined by stratified squamous epithelium, with dermis showing adnexal structures including hairs surrounded by collagen. Group 2 (induction of oral mucositis) at 15th day represents the hyperplastic squamous epithelium, dermis shows dense collagen with scattered inflammation with loss of adnexal structures. Group 2 at 18th day represents irregular healing with denucleation of squamous epithelium with scattered inflammation. Group 3 (induction of oral mucositis and treatment with sucralfate paste) at 15th day represents incomplete healing with irregular regeneration of squamous epithelium, loss of adnexal structures with dense collagen and scattered inflammation, it indicates that the process of healing of oral mucositis has started due to the treatment given from 12th day. Group 3 at 18th day represents complete healing due to the continuous application of sucralfate paste for a week.

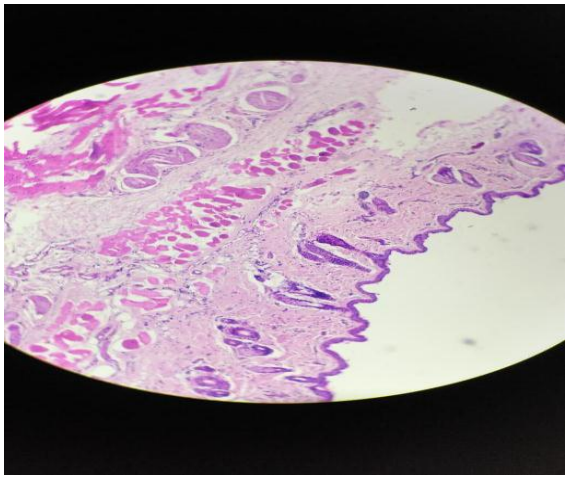

Fig. 13: Histology of Group 1 (Magnification 100 X).

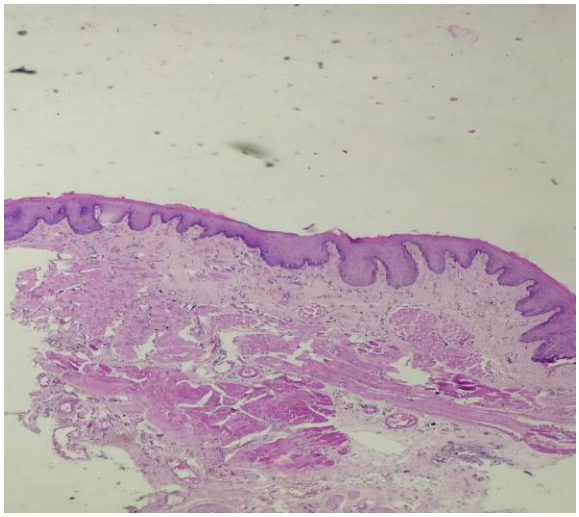

Fig. 14: Histology of Group 2 at 15th day (Magnification 100 X).

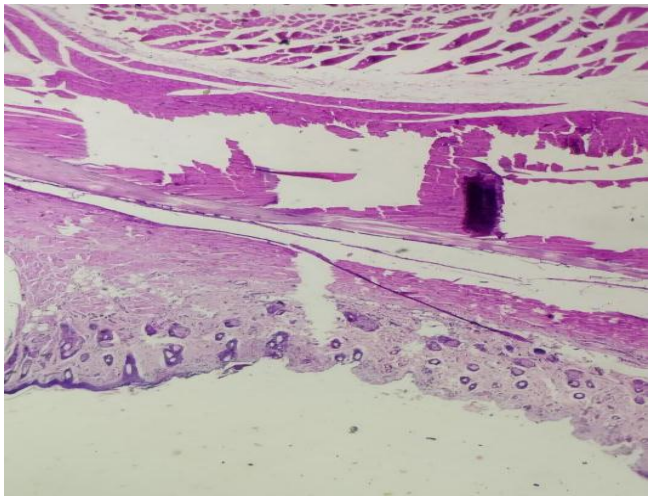

Fig. 15: Histology of Group 2 at 18th day (Magnification 100 X).

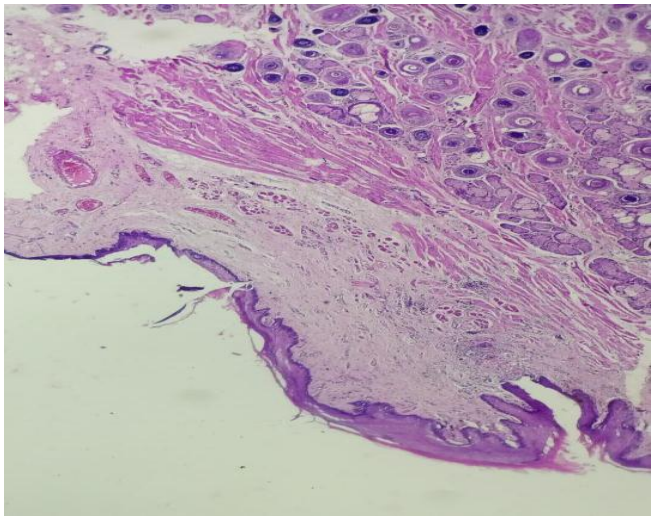

Fig. 16: Histology of Group 3 at 15th day (Magnification 100 X).

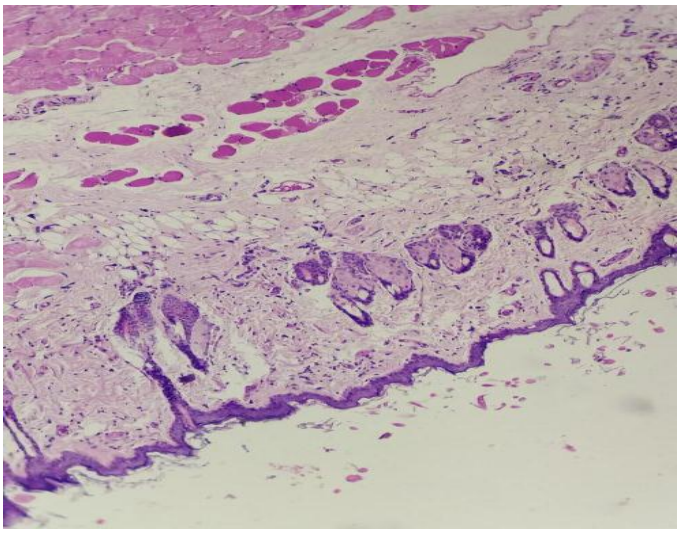

Fig. 17: Histology of Group 3 at 18th day (Magnification 100 X).

CONCLUSION

Oral mucositis is the severe complication which often results from chemotherapy. Providing treatment for oral mucositis can delay cancer treatments which affects the health of the patients. Sucralfate paste prepared by polymerization method prevent and treat oral mucosa by forming a physical barrier, it is easy to apply and increases the contact time of the paste with oral mucositis. *In-vitro* and *in-vivo* results of the present study suggest that sucralfate paste is effective in treating oral mucositis, ultimately it can increase the survival of the patients.

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