

ORIGINAL RESEARCH ARTICLE

Histopathological Effect of Mucoadhesive Herbal Gel on Tobacco Carcinogenicity

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ABSTRACT

In the present work, stress was given for improvised local action of the drug with the addition of mucoadhesive polymer in the formulation. Oral mucoadhesive semi solid gel was prepared for the treatment of oral sub mucous fibrosis, which provides effect for extended periods of time. Curcumin was taken as a model drug as it exhibits profound antitumeric & antimutogenic activity. The semisolid preparation was comprising of stabilizer like sodium metabisulphite, muco retention / mucoadhesive polymer NaCMC and was subjected for various physicochemical parameters like pH, spreadability, drug content uniformity, extrudability, viscosity & I.R. studies. *In-vitro* drug release studies were carried out in phosphate buffer (6.4 pH). Stability studies were also done at room temperature for a period of eight weeks. The formulation showed good *in-vitro* release and good adhesion to oral mucosae. IR studies showed that there was no drug-excipient interaction. The *in-vivo* studies were carried out in two phases using 18 mice with the permission of ethical committee under the supervision and help of staff, Department of Pathology, M.R. Medical College, Gulbarga. In first phase oral sub mucous fibrosis was induced in mice using marketed Gutkha preparation and formulating into a mucoadhesive gel form and applying to mice oral mucosa with the help of cotton bud for a period of 6 months. In second phase, treatment was carried out following the above method using curcumin formulation. The tissue samples collected for 1, 3 & 6 months induction period & 1, 3 & 6 months of treatment period on 6 months oral sub mucous fibrosis induced mice. Histopathological observations reported that there was considerable induction of oral sub mucous fibrosis and excellent treatment results on curcumin usage. The results of the present study of mucoadhesive semi-solid drug design for the treatment of oral sub mucous fibrosis will be useful for drug industry for the benefit of patients suffering from oral sub mucous fibrosis.

Key words: Mucoadhesive, semi solid preparation, curcumin, oral sub mucous fibrosis.

INTRODUCTION

The habit of chewing tobacco based products like Pan Masalas, Gutkha etc., contains many toxic chemicals which irritate the delicate skin of oral mucosa. This causes oral sub mucous fibrosis (OSMF) a pre-malignant fibrotic lesion of buccal region characterised by dense bands of collagen in the juxta-epithelia proceeded by inflammation and in later stages leads to oral cancer. In India 30-40% patients of oral inflammations are suffering with OSMF. In early stages vesicles or fibrous bands are present on the labial mucosa associated with pigment changes. In later stages, the mucosa become stiff, causing difficulty in opening the mouth. Histologically, the mucosa varies from atrophic to normal. A characteristic feature is a prominent sub epithelial eosinophilic band. The

juxtaepithelial connective tissue is amorphous and non bundular as against the normal undulated bundular collagen^[1,2,3]. The main cause for Oral submucous fibrosis are chewables like gutkha, tobacco, pan masalas, areca nut.^[4] A thorough literature survey has been carried out on the effect of turmeric oil and turmeric oleoresin on cytogenetic damage in patients suffering from OSMF^[5]. *In vitro* studies on the effect of alcoholic extracts of turmeric, turmeric oil and turmeric oleoresin, on the incidence of micronuclei in lymphocytes from normal healthy subjects showed that the test compounds did not cause any increase in the number of micronuclei as compared with those found in untreated controls^[5]. The effect of placenta extract in

management of Oral submucous fibrosis and stated that there was significant improvement in mouth opening, colour of oral mucosa and reduction of fibrous bands^[6]. A simple and easy method of estimation of curcumin based on the solubility of curcumin in methanol is developed by^[7]. Prepared and characterized curcumin gels using a bioadhesive polymer like pluronic F-127 for local application as topical therapeutic system^[8]. Zinc sulphate gel using a Sodium Carboxy Methyl Guar as oral gel for mouth ulcers formulated by^[9]. Buccal mucoadhesive films and mucoadhesive gels of captopril was prepared using Hydroxy propyl methyl cellulose, ethyl cellulose and carbopol. The drug release pattern was higher with formulations containing carbopol^[10], tetracycline hydro chloride gels were designed and evaluated by^[11]. The tetracycline gels formulated with hydroxy propyl methyl cellulose and carbopol showed increase in drug release with increase in polymer concentration^[11]. There is no effective treatment for OSMF and there is a need for drug research for its treatment in any type of dosage form. In the present study an attempt has been made to develop mucoadhesive semisolid preparations of Curcumin for oral application directly on to the inflamed site to produce local action, using mucoadhesive hydrophilic polymer like Sodium carboxy methyl cellulose (NaCMC)^[12].

MATERIAL AND METHODS

Curcumin was procured from Sami Labs, Bangalore, Glycerin from Ranbaxi Lab Ltd. Chandigarh, Sodium carboxy methyl cellulose was purchased from S. D. Fine Chem. Ltd., Mumbai, Sodium meta bisulphite and methanol from Qualigens Mumbai.

Preparation of formulation

The semisolid formulation was prepared using Sodium carboxy methyl cellulose, as base. Prehydrated polymer samples for 12 hours were dissolved in 85 ml of distill water on constant stirring for about one hour. Then added 15 ml of ethanolic curcumin solution. On continues stirring, glycerine, sodium meta bisulphite were dissolved in the above Polymer-Drug solution.

Evaluation of physicochemical parameters

The prepared formulation were subjected for various physicochemical parameters such as spreadability, extrudability, pH, viscosity, Mucoadhesive, drug content estimation^[13,14].

Spreadability

Spreadability was determined by an apparatus suggested by Muttimer *et al.*, which was

suitability modified in the laboratory and used for the study

Extrudability

The formulation under study was filled in a clean, lacquered aluminum collapsible one-ounce tube with a nasal tip of 5 mm opening extrudability was then determined by measuring the amount of ointment, cream and gels extruded through the tip when a constant load of 1 Kg. was placed on the pan were collected and weighed. The percentage of ointment, cream and gel extruded was calculated: recorded and grades were allotted (+++ Good; ++ Fair; + Poor). (Table 2)

Determination of Viscosity Formulation under study was subjected to viscosity studies. Instrument used to measure viscosity is Brookfield digital viscometer (Table 2).

Determination of pH

Weigh accurately 5 ± 0.1 gm. of the cream in a 100 ml. beaker, add 45 ml. of water and dispersed the cream in it. Determine the pH of the suspension at 27°C using the pH meter (Table 2).

Determination of Drug Content Uniformity

Drug content uniformity was carried out by taking 5 gm sample of prepared formulation and subjected for analytical assay to calculate the drug present in the sample using UV spectrophotometer at λ_{\max} 430 nm (Table 2). The drug content was uniform in all formulations.

Mucoadhesive Studies

The glass plates are coated with the polymer and suspended from a microbalance. The glass plate is immersed in a temperature controlled mucous solution. The force required to pull the plate out of solution is determined under constant experimental conditions. A number of methods use liquid adhesive mass for evaluation. Duration of mucosal adhesion i.e., the time span required until the adhesive patch completely loses its adhesive contact with the mucosa was measured HEC and hydroxy propyl cellulose possess superior mucosal adhesion in human subjects and time span value are 30 and 15 minutes respectively. The results are given in the (Table 2)^[13].

Drug polymer interaction studies

The studies were carried out using IR method with the help of Perkin-elmer 1615 spectrophotometer to check the possible drug polymer interaction (Fig 1)

Evaluation of Drug Release

Release of the curcumin from semisolid preparation was studied by applying the permeation apparatus as directed by Fitter *et al* (Table 4).

In-vivo Studies

The *in-vivo* studies were carried out in mice with the permission of ethical committee under the supervision and help of staff, Department of Pathology, M. R. Medical College, Gulbarga. The *in-vivo* studies were carried out in two phases using 18 mice.

- ❖ Induction of OSMF in animals for a period of six months.
- ❖ Treatment of OSMF on the induced animals.

Induction of OSMF

18 (eighteen) Swiss male albino mice weighing 25 - 30 gms were selected for the experimental design in the present work OSMF was induced with the causative ingredients of marketed brands of gutkhas. The gutkha powder was pulverized with the help of mortar and pestle and passed through Sieve No. 200. Mucoadhesive gel formulations containing 1% gutkha powder prepared in the laboratory were applied with the help of cotton bud on to the buccal mucosa of the animals for a period of 6 months. During the induction period the animals were without water and food for a period of 6 hours and other times with regular food and water. To study the effect of induction a punch biopsy technique was used by sacrificing the animals using skin punch biopsy forceps (No. 5). The biopsy sample of buccal mucosa collected in normal saline vials of 3 animals was subjected for histopathological slide preparation and study of observation. The similar procedure was followed to check the induction after 3 months and 6 months. A biopsy sample of buccal mucosa of 3 healthy animals, were collected and set aside for comparative purpose.

Ingredients of Gutkha

Betelnuts, Catechu, Lime, Cardamom, Menthol, Tobacco, Natural perfumes, Sandal oil species & flavours (**Table 1**).

Procedure details

85 ml of distilled water was taken in 250 ml glass beaker. Then add the polymer, glycerine and the preservative (Sodium metabisulphite) and mix with a glass rod. Cover the beaker with a glass plate and keep aside for 24 hrs for hydration of the polymer. Then add gutkha powder to 15 ml of water. This solution was added slowly to the hydrated base and mixed using a Rem stirrer at 100 rpm.

Treatment

After six (6) months of induction study. The remaining nine animals were tested for the purpose of treatment of OSMF, 1% curcumin muco adhesive gel prepared in the laboratory was

used. The curcumin gel was applied on to the oral cavity of buccal mucosa in mice with the help of cotton bud and the procedure followed for application as used in induction method. For histopathological observations of treatment, the biopsy samples were collected on 3 animals each after 1 month, 3 months & 6 months. Unlike in induction process, the slides of smears of the biopsy sample were processed for comparative evaluation of treatment.

Stability studies

The formulation was then packed in the collapsible tube and stored at room temperature for 8 weeks and studied for viz., spreadability, extrudability, pH, drug content, viscosity (**Table 3**).

RESULTS AND DISCUSSION

The gel was subjected to physical evaluations such as viscosity, extrudability, spreadability, pH, drug content uniformly and results are shown in Table-2. During our physico-chemical evaluation studies the formation was within pH range. Drug content estimation, drug present in formulation was found to be 99.63. The formulation showed good mucoadhesion for 31 minutes. Mucoadhesive curcumin gel was evaluated for drug polymer interaction by infrared spectral studies. After comparing the spectra i.e., absorption bands of pure drug with the spectra of formulation, the absorption bands of the pure drug were retaining in the formulation without undergoing any interaction with the polymers.

In-vitro drug release from curcumin gel was studied, and *in-vitro* data obtained has been shown in table no 4. In the formulation at the end of 120 min, percent cumulative drug release was 24.21%. In our present investigation of stability studies, the formulation did not segregate, ferment or physically deteriorated during normal condition of storage and use. When stored at room temperature for a period of 8 weeks the formulation did not undergo phase separation of gassing formulation or otherwise deterioration aesthetically (Table 3). The formulation was then planned for *in-vivo* studies using mice as model animal. The present study tries to focus the array of histomorphological changes in oral mucosa of albino mice after oral application of gutkha and histomorphological changes in already OSMF induced albino mice after oral application of curcumin and to see whether curcumin can heal OSMF^[15]. In first phase of histopathological studies of OSMF induction in mice, a gross change of mucosa is observed and increased

significance seen with the use of gutkha gel from 1 month application to 6 months applications. In second phase of treatment part of OSMF using prepared curcumin semi-solid preparation the encouraging results were observed. There is a marked reduction (more than 50%) of OSMF seen from the histopathological studies on the

specimen samples taken after 1 month, 3month and 6months(Fig 2)

Table 1: Formula used to prepare muco adhesive semi solid preparation gutkha of marketed product

Ingredients	Quantity
Gutkha (Seive No. 200)	1.0 gm
Polymer (NaCMC)	4 gm
Glycerine	2.0 gm
Sodium Meta bisulphite	0.5 gm
Distilled water q.s. (ml.)	100 gm

Table-2: Characterisation of prepared formulations

Spreadability (Sec)	Extrudability	Viscosity (CPS)	pH	Drug Content (%)	Duration of Mucosal Adhesion (min)
14.11	+++	2.6 x 10 ⁵	6.7	99.63	31

Table-3: Stability studies data

Storage Temp	Time of Analysis	Spreadability (Sec)	Extrudability	pH	Viscosity (CPS)	Drug Content (%)
Room Temperature	1 st Week	14.12	+++	6.7	2.6 x 10 ⁵	99.63
	2 nd Week	14.12	+++	6.7	2.6 x 10 ⁵	99.63
	3 rd Week	14.11	+++	6.6	2.5 x 10 ⁵	99.62
	4 th Week	14.11	+++	6.5	2.5 x 10 ⁵	99.63
	5 th Week	14.11	+++	6.4	2.4 x 10 ⁵	99.62
	6 th Week	14.12	+++	6.4	2.4 x 10 ⁵	99.62
	7 th Week	14.10	++	6.4	2.5 x 10 ⁵	99.63
	8 th Week	14.10	++	6.4	2.5 x 10 ⁵	99.62

Table 4: In-vitro release of Curcumin (1%) from Mucoadhesive Semisolid preparations containing NaCMC

S No	Time	Absorbance	Concentration	Cumulative Drug Release	Percentage Cumulative Drug Release
1	0	0	0	0	0
2	10	0.350	3.75	0.375±0.035	3.75
3	20	0.671	7.21	0.739±0.025	7.39
4	30	0.959	10.08	1.044±0.041	10.44
5	40	1.228	13.26	1.376±0.040	13.76
6	50	1.458	15.47	1.613±0.032	16.13
7	60	1.685	17.64	1.841±0.025	18.41
8	70	1.792	19.23	2.011±0.045	20.11
9	80	1.919	20.38	2.134±0.034	21.34
10	90	1.968	21.19	2.220±0.040	22.20
11	100	2.006	21.51	2.256±0.045	22.56
12	110	2.102	21.62	2.269±0.035	22.69
13	120	2.158	23.13	2.421±0.040	24.21

* Each reading is a replicate of three determinations.

* 5 gm. of semi-solid preparation contains 50mg of curcumin.

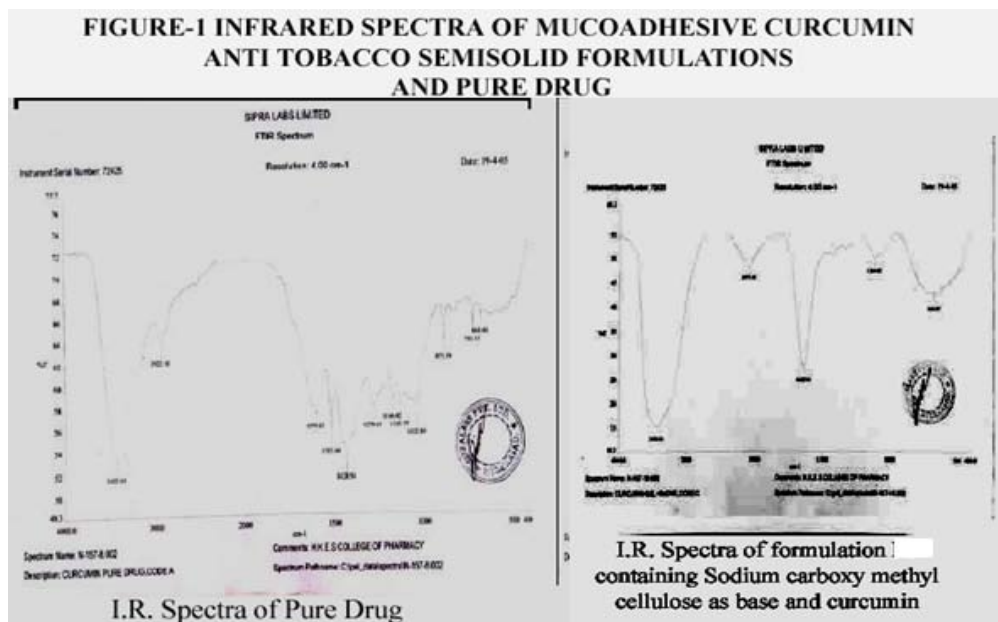
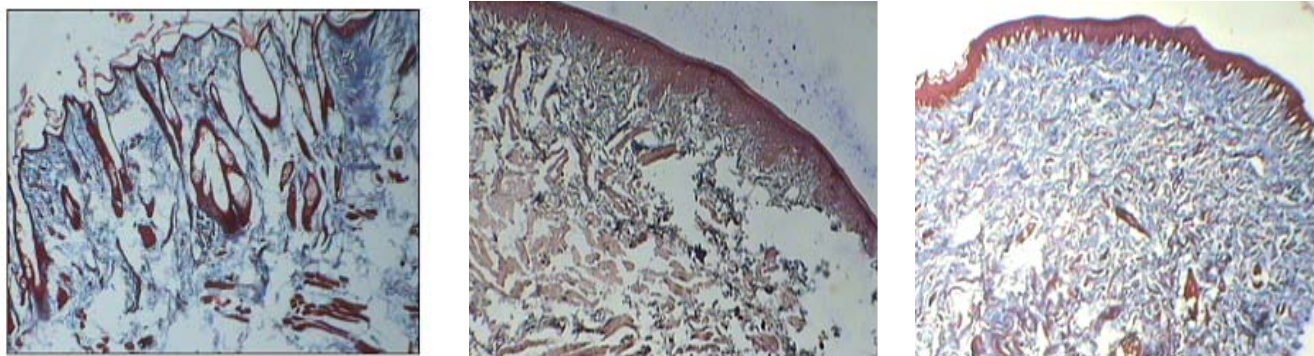


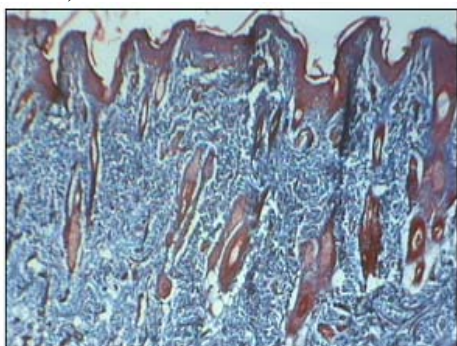
Fig 2: Section of oral mucosa of mice after induction of OSMF with Gutkha at different periods of time



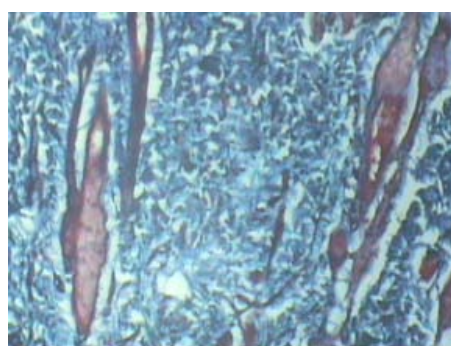
Special Stain – Masson’s Trichrome for Collagen Fibers (Normal)

Gutkha – 1 Month (10x) Mild Increase

Gutkha – 3 Months (10x) Moderate Increase

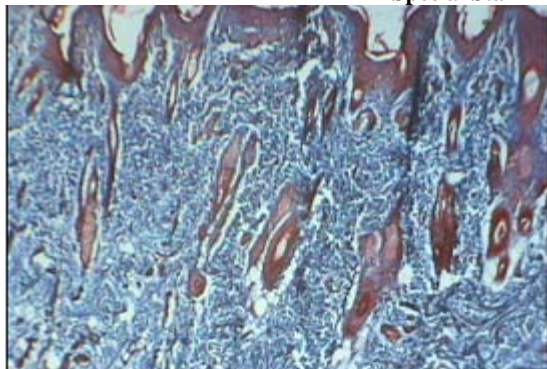


Gutkha – 6 Months (10x) Marked Increase

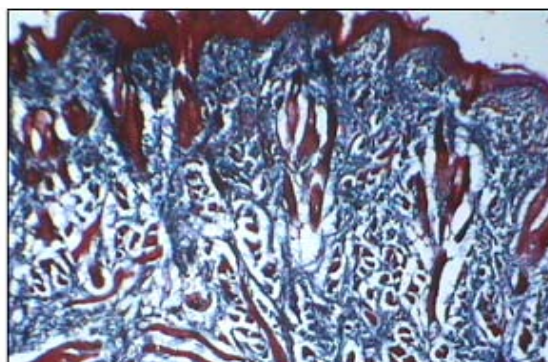


Gutkha – 6 Months (40x) Marked Increase

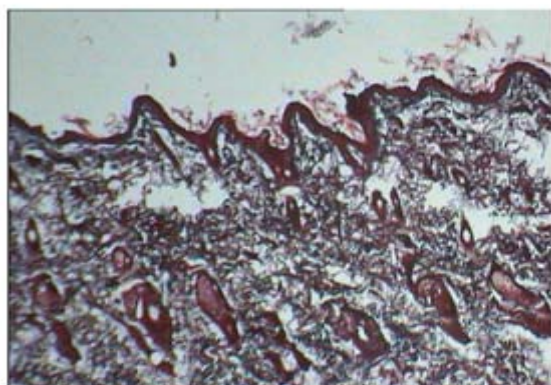
Fig 3: Section of oral mucosa of mice after treatment of OSMF with turmeric at different periods of time
Special Stain – Masson’s Trichrome for Collagen Fibers



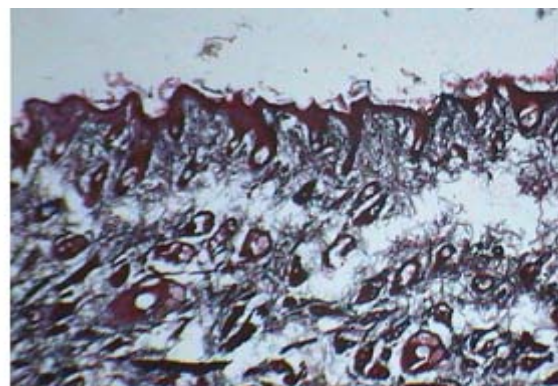
Gutkha – 6 Months (10x) Marked Increase



Turmeric – 1 Month (10x) Mild Decrease



Turmeric – 3 Months (10x) Mod Decrease



Turmeric – 6 Months (10x) Minimal Collagen Tissue

CONCLUSION

The results of the present study of mucoadhesive semi-solid drug design for the treatment of OSMF will be useful for drug industry for the benefit of patients suffering from OSMF.

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REFERENCES

1. Harsh Mohan, "The aetiology of Oral sub mucous fibrosis" "A text book of pathology" 4th edition, J P Publication, 2000; New Delhi, 325 pp.
2. Bailey & Love's, "The aetiology of Oral sub mucous fibrosis" "Short practice of surgery" 2nd edition, Arnold Publishers, 2000; 639 pp.
3. Canniff J P Harvey, "The aetiology of Oral sub mucous fibrosis", *Int. J of Oral surgery*, 1981; 10 (1): 163-67 pp.
4. Huang S, Ling T, Wu H, "Experimental Study on aqueous areca nut extracts inducing Oral submucous fibrosis in rats", *Carcinogenesis*, 1997; 15(2):91-96pp.
5. Hastak K, Lubri N, Jakhi SD, More C, John A, Bhaisa SD, Bhide SV, "Effect of turmeric oil and turmeric oleoresin on cytogenetic damage in patients suffering from oral submucous fibrosis", *Cancer-Lett*, 1997; 116(2): 265-9.
6. Katharia S K, Singh S P, Kulshreshtha V K, "The effect of placenta extract in management of Oral submucous fibrosis", *Ind. J of Pharmacology*, 1992; 24(6): 181-83.
7. Krishna Prasad NS, Sarasija Suresh, "Spectrophotometric estimation of curcumin" *Indian Drugs*, 1997; 34(4):227-28pp.
8. Sarasija Suresh, Shobha Rani R Hiremath, Praveen S, Aney Thomas, "Preparation and Evaluation of pluronic F-127 gels of ketenazole and netekonazole", *Indian Drugs*, 1999; 36(5):326-28pp.
9. Dr. Paranjothy K L K, D Thampi, "Application of Sodium Carboxy methyl Guar as a base in zinc sulphate gel formulation", *Indain Drugs*, 1995; 32(4): 186-88pp.
10. Pandey S, Pai M, Singh U V, Udupa N, "Mucoadhesive formulations of Captopril", *Pharmag*, 2001; 13(1,2): 12-15pp.
11. Uma Devi, Ganesh M, Mohanta G P & Manavalan R, "Design & evaluation of tetracycline HCl gels", *Indian Drugs*, 2002; 39(10):552-53pp.
12. Wade Aniley & Weller J Paul Ed, "Hand book of practical excipients profile of NaCMC". The Pharmaceutical Press London, 1994; 78pp.
13. Rameshacharya Bala R, Vani G. & Rao Madhusudhan Y, "In-vitro & in-vivo adhesion testing of mucoadhesive drug delivery system", *Drug development & industrial pharmacy*, 1999; 25 (5): 685-90pp.
14. Subrahamanyah CVS, Reddy Shreenivas, "Buccal Mucoadhesive Drug Delivery System", *Pharmag*, 1998; 7:91-95pp.
15. Culling FA, "A book of Histopathological & Histochemical techniques", Butter work & co. publication, Great Britain, 3rd edn. 1975; 5-50pp.