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A Novel Mucoadhesive Biomaterial from Natural Source Glycine Max

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ABSTRACT

Our objective was to isolate a novel biomaterial from the seed of Glycine max, a member of the Leguminoceae family and native of East Asia. In India it is available in Madhya Pradesh. The biomaterial was isolated by the non-solvent addition method and its colour, texture, solubility and chemical and IR spectral properties were determined. The biomaterial swelling index, colour change point, and acute animal toxicity were also studied. The mucoadhesivity of the biomaterial was determined by the shear stress, Park and Robinson, and rotating cylinder methods, and the results were compared with those of the standard polymers sodium carboxymethyl cellulose and hydroxypropylmethyl cellulose. The research study revealed that the biomaterial from G. max exhibits promising inbuilt mucoadhesion and good mucoretentability. The mucoadhesion of the biomaterial was also confirmed by IR spectra showing carboxyl and hydroxyl groups. Hence the isolated biomaterial from the G. max can serve as a powerful natural mucoadhesant and may be used to develop mucoadhesive transmucosal drug delivery systems.

Keywords: Natural biomaterial, transmucosal delivery, Leguminoceae, FTIR, Glycine max

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INTRODUCTION

Mucoadhesion is the relatively new and emerging concept in drug delivery. Mucoadhesive polymers facilitate the mucoadhesion by their specific properties. The focus of pharmaceutical research is being steadily shifted from the development of new chemical entities to the development of novel drug delivery system (NDDS) of existing drug molecule to maximize their effective in terms of therapeutic action and patent protection. [1] The development of NDDS has been made possible by the various compatible polymers to modify the release pattern of drug. [2, 3] In the recent years the interest is growing to develop a drug delivery system with the use of a mucoadhesive polymer that will attach to related tissue or to the surface coating of the tissue for the targeting various absorptive mucosa such as ocular, nasal, pulmonary, buccal, vaginal etc. [4] If they are biocompatible and biodegradable this provides added advantages for formulating various controlled release pharmaceutical formulations and avoids patient noncompliance, especially for chronically ill patients. The advantages of such materials include their natural origin, ready availability, low cost, biodegradability, and capability of a multitude of chemical modifications. The majority of natural mucoadhesive agents are polysaccharides or proteins. Seeds of Glycine max furnish one of the world's most important sources of oil and protein. Unripe seeds are eaten as vegetable and dried seeds eaten whole, split or sprouted. The plant has been traditionally used as a remedy for the proper functioning of the bowels, heart, kidney, liver, and stomach. The objective of this study is to isolate the biomaterial from the seed of Glycine max and to determine its intrinsic mucoadhesive and mucoretentive properties.

MATERIALS AND METHODS

Seeds of Glycine max were obtained from the local market. Acetone, sodium dihydrogen orthophosphate, potassium dihydrogen orthophosphate, and sodium hydroxide were purchased from Qualigen Chemicals Pvt. Ltd. Double distilled water was prepared from the institutional laboratory. All chemicals used were of analytical grade.

Extraction of mucoadhesive biomaterial from Glycine max

Extraction of the biomaterial was performed by first soaking 100 g seeds of G. max with 500 ml of distilled water for 24 h in a refrigerator at 5 °C. The mixture was grounded into thick paste and then filtered with equal volume of water. The filtrate was centrifugation at 4000 rpm for 120 min and collect the supernated cream layer. The biomaterial was recovered from the extract via precipitation with 3 volumes of acetone. The precipitated biomaterial was washed repeatedly with acetone, collected, purified by dialysis, and dried at 50-60 °C under vacuum for 12 h. The dried biomaterial was pulverized and passed through a 200 mesh sieve and stored in desiccators.

Physicochemical characterization

The texture, solubility, pH of 1% biomaterial solution, swelling factor, viscosity, colour changing point, and UV and FTIR spectra for the biomaterial were measured. [5] Elemental analysis and thin layer chromatography were also performed.

Assessment of mucoadhesive properties

The mucoadhesive property of G. max seed extract was determined in vitro by the shear stress method [6], ex vivo by the Park and Robinson method [7], and rotating cylinder method [8]. The biomaterial was subjected to a shear stress study for in vitro assessment of its adhesive strength in terms of weight required for breaking adhesive bonds between polymer and glass plate in a specified contact time of 5, 10, 20, or 30 min period with concentrations of 0.5%, 1%, 2%, 3%, or 5% w/v of the natural mucoadhesive extract and compared with the standard polymer NaCMC 1% and Carbapol 2%. With the Park and Robinson method, the biomaterial was punched into a small circular bioplate of thickness 0.2 mm using a hydraulic pelletizer and the force required to detach the bioplate from the mucosal surface was determined and compared with that of the standard polymers sodium carboxymethyl cellulose (NaCMC) and carbapol. The average of 6 readings was registered. With the rotating cylinder method, the stainless steel rotating baskets were covered with a thin layer of aluminium foil. A freshly excised goat soft palate tissue was secured onto the aluminium foil surface around the rotating cylindrical basket. The compressed bioplate was placed on the mucosal surface. It was then put in a flask containing 900 ml of phosphate buffer at pH 6.8 at 37± 2 °C. The cylindrical baskets were rotated at 100 rpm. Every 30 min the machine was stopped and checked for dislodgement or disintegration of the bioplate from the mucosal surface. The results were compared with the standard polymers NaCMC and carbapol. The average of 6 readings was registered.

Acute toxicity study of the extract

The biomaterial was evaluated for acute toxicity study. The study protocol was approved by the Institutional Animal Ethical Committee. The procedure followed was as per OECD 423 guidelines. Two groups of 6 albino unisex rats, one for test and other for control, were used for the study. The animals were provided with free water ad libitum. The study was performed by administering the dried biomaterial at 2 g/kg body weight for the test group animals and the acute toxicity study was evaluated for a period of 14 days by observing body weight, changes in the skin, corneal reflex, respiratory rate, autonomic symptoms, salivation, diarrhoea, lethargy, sleep, somatomotor, behavioural patterns, and convulsions.

RESULTS AND DISCUSSION

Novel biomaterial from Glycine max was isolated by simplified economical process the yield was 1% per 100gms. The biomaterial obtained was of brownish to dark brown color with a colour changing point of 167-170˚C. The biomaterial showed positive tests for the presence of

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proteins and carbohydrates (Table 1 and Table 2). The functional groups test showed the presence of ketone, aldehyde, and alcoholic hydroxl groups. Swelling factor (1 g) was 1.9 ml and UV spectra showed a λ_{max} of 251 nm. The FTIR spectra of the biomaterial was performed (Fig. 1) and the interpretation of the IR spectra is given in (Table 3). The presence of carboxyl and hydroxyl groups in the biomaterial is the key to its mucoadhesive properties. Elemental analysis showed the presence of carbon 28.60%, hydrogen 4.70%, and nitrogen 1.32%. Thin layer chromatography showed the presence of galactomannan.

Table 1: Physical properties of biomaterial

S. No.	Physical property	Inference
1.	Colour	Brownish to dark brown
2.	Odour	Odourless
3.	Taste	Characteristic
4.	Colour changing point	167-170°C

Table 2: Chemical identification tests of the biomaterial

S. No.	Chemical test	Observations	Inference
1.	Fehlings test	Positive	Carbohydrates present
2.	Benedicts test	Positive	Carbohydrates present
3.	Molischs test	Positive	Carbohydrates present
4.	Ninhydrins test	Positive	Protiens present
5.	Biurets test	Positive	Protiens present

Table 3: Interpretation of the IR spectra

S. No.	Wave number (cm^{-1})	Inference
1.	3738.45	O-H (stretching)
2.	3156.63	C-H (stretching)
3.	2358.65	C=C (stretching)
4.	1651.44	C=O (stretching)
5.	997.09	C-O (stretching)
6.	632.14	C-H (Def)

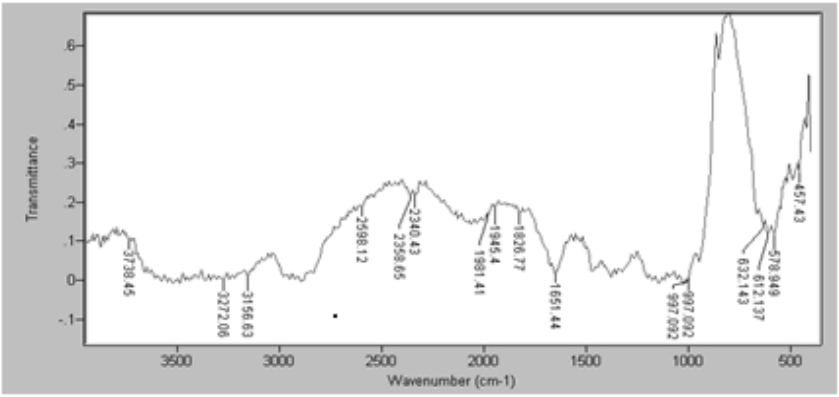

Fig 1: FTIR spectra of the natural mucoadhesive extract of Glycine max.

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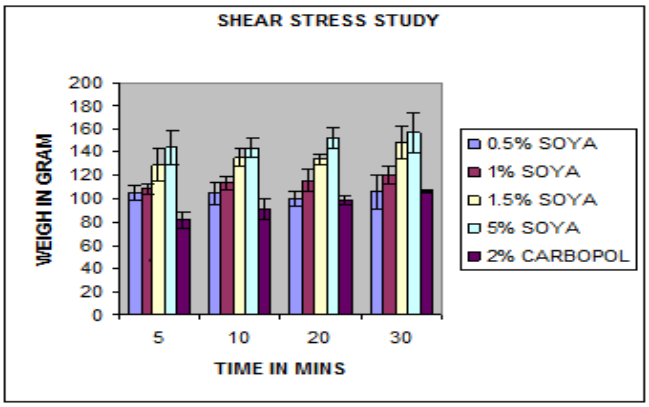

Fig 2: Comparison of bioadhesive property of the natural mucoadhesive extract with synthetic polymers 2% Carbopol as determined by shear stress method.

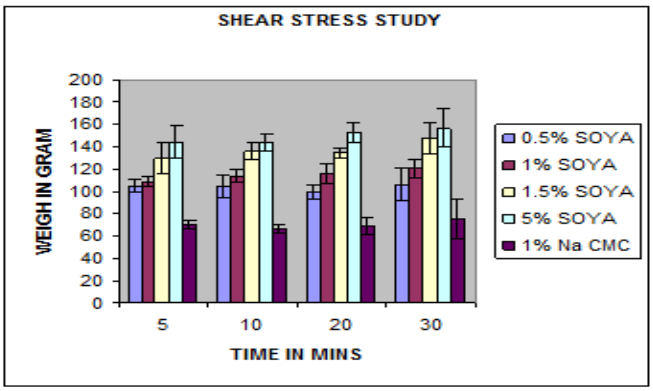

Fig 3: Comparison of bioadhesive property of the natural mucoadhesive extract with synthetic polymers 1% NaCMC as determined by shear stress method.

Assessment of mucoadhesive property

A 0.5% w/v solution of the mucoadhesive extract of exhibited excellent bioadhesive strength (Fig. 2 and Fig. 3). Further increment in the biomaterial concentration did not affect its bioadhesiveness. This is probably due to loss of hydration by evaporation that increases the mucoadhesive strength. The macromolecules containing numerous hydrogen bond forming groups (e.g., hydroxyl, carboxyl groups) show the most promising mucoadhesivity.

The ex vivo mucoadhesivity of the extracted biomaterial of G. max by Park and Robinson method showed that the biomaterial possesses a promising mucoadhesivity in comparison to NaCMC and was found to be similar to that of carbopol. The process of mucoadhesion has been proposed to begin with the establishment of an intimate contact between the mucoadhesive polymer and mucus gel. [9] The plausible mechanism of its mucoadhesive property may be the interaction of mucus with carboxyl or hydroxyl groups of the biomaterial. The role of surface

energy thermodynamics in mucoadhesion has been considered vital [10] for the mucoadhesive strength exhibited by the biomaterial extract of G. max.

The rotating basket method revealed that the biomaterial had a promising mucoretentability which was found to be higher than that of NaCMC or carbapol. This is due to the fact that G. max having high molecular weight exhibited higher adhesion and better mucoadhesion than the synthetic polymers (NaCMC and carbapol) at the same concentration. This may be due to the presence of numerous disulphide bridges and carboxyl and hydroxyl groups, which adopt more favourable macromolecular conformation, and accessibility of its hydrogen-binding groups. Carbapol and NaCMC, being cellulose derivatives, form weaker bonds with mucus, which may be due to either a decrease in available hydrogen binding sites or unfavourable entanglement with the mucus.

Acute toxicity study of extracted biomaterial

The biomaterial was devoid of signs of toxicity in animals tested. This may be due to the edible nature of the G. max seeds.

CONCLUSIONS

It can be concluded that G. max seed extract is a better mucoadhesive agent than carbapol and NaCMC with respect to inbuilt mucoadhesive and mucoretentive properties. Since this natural mucoadhesive agent is edible, it is easily biodegradable and not an allergen and may provide an alternative to conventional synthetic and natural mucoadhesive agents.

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