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Preparation, characterisation of N-aryl chitosan and screening for its hypocholesterolemic activity

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ABSTRACT

The present research work entails synthesis and pharmacological screening of chemically modified N-aryl chitosan derivatives. Chitosan is an amino polysaccharide obtained from natural origin and it has attracted attention because of its unique physicochemical characteristics and biological activities. It has great pharmaceutical futuristic potential with unusual extent of possibilities for structural modifications to impart desired functions. This chemically modified chitosan can be used as potent hypocholesterolemic agent. Various aldehydes can be used for the chemical modification of chitosan, where aldehyde attached to the free amino group of chitosan polymer imparts different physicochemical properties, not exhibited before modification. An imine formation as an intermediate is then reduced to N-aryl derivative of chitosan. It was characterised by IR and NMR. The hypocholesterolemic mechanism of chitosan was investigated in male wistar rats. Animals were divided into 6 groups (n = 6): a normal untreated control group (UC), a high-fat control group (PC), one standard control (SC) and 3 modified chitosan groups (CSA1, CSA2 and CSA3). The doses of standard chitosan and modified chitosan were given at the beginning for 12 days. Later it was preceded by high fat induction. The results showed marked decrease in total serum cholesterol of rat's in-vivo that refers to modified chitosan treatment.

Key words: N-Aryl chitosan, Hypocholesterolemic activity, Modified chitosan, Schiff base.

INTRODUCTION

Coronary heart disease is the prime cause of death in the world. The factor includes excessive intake of calories and fats and the accumulation of adipose. In recent time, many articles reported on how to decrease plasma lipid concentrations as well as the fat absorption in the intestinal tract to reduce diet-related chronic disease. Dietary fiber such as pectin and psyllium shows some potent hypolipidemic effect. Chitosan, a polymer of glucosamine, can be defined as a dietary fiber chemically and physiologically because it cannot be degraded by the digestive enzymes of human. Additionally, it is the only abundant polysaccharide derived from animals, and its cationic characteristics are different from other dietary fibres. It is natural, non-toxic and rising verification indicate that it exhibits a noticeable hypolipidemic activity that would shrink the risk of cardiovascular diseases. Chitosan has potent fat-binding ability in vitro. Maezaki et al was the first to report the hypocholesterolemic effect of chitosan in humans and establish that chitosan effectively decreased plasma lipid level and had no side effect.[1-4]

An attempt was done in the chemical modification of chitosan to assess their hypocholesterolemic activity in comparison with non-modified chitosan. The modified chitosan derivatives were synthesized by using various aromatic aldehydes which initiate formation of imines as well. The structural characterisation of this modified N-aryl chitosan was done by IR and ^{1}H-NMR spectroscopic methods.

The result suggests that the mechanism involved in lowering cholesterol levels in their property of resins. Chitosan acts as a weak anion exchange resin and exhibits an extensive viscosity in vitro. Either of these properties of chitosan could arbitrate its hypocholesterolemic effect. Thus, the anion exchange property of chitosan would appear to be preferred as a justification for its hypocholesterolemic properties.

In addition, with the longer time needed for chitosan treatment, the retardation of body weight gain in rats was clear. This might signify that chitosan could be used as weight loss agent for both healthy and obese humans, which might be because of the binding of lipid in the gastrointestinal tract thus reducing fat absorption.

MATERIALS AND METHODS

The synthesis of substituted N-aryl derivatives of chitosan was carried out by following step:

1. Synthesis of N-aryl derivative of Chitosan [5-13**]:**
Chitosan was dissolved in a solution of acetic acid in methanol. Aldehyde was added to the solution and stirred at room temperature for 4 Hrs. After standing the mixture for 3 Hrs, $NaBH_4$ was added and stirred at room temperature for 24 Hrs. The solution was then made alkaline by addition of sodium hydroxide. The precipitate formed after addition of sodium hydroxide is separated by filtration, dried at 60^0C in oven for 24 Hrs.

2. Characterisation of synthesized derivative [5-13**]:**
Infrared (IR) and Proton Nuclear Magnetic Resonance (^{1}H-NMR) were used to confirm the structures of all the synthesized compounds. IR spectra were recorded on a Jasco FTIR-410 spectrophotometer using KBr pellets. ^{1}H-NMR spectra were recorded on varian mercury YH-300 using CF_3COOD as solvent at 300 MHz. TMS was used as an internal reference standard for relative proton chemical shift.

3. Hypocholesterolemic activity [1, 3, 14**]:**
Animals and diet:
Male wistar rats weighing 150 ± 220 g were purchased & they were individually housed in metabolic cages in a controlled environment (25°C ± 1°C; 50%-60% relative humidity; 12-hour light-dark cycle with lighting from 8:00 AM to 8:00 PM). All animal protocols were approved by the institutional animal care. Rats were allowed free access to food and water. Rats were randomly divided into 6 groups (n = 6): normal untreated control group (UC), a high-fat control group (PC), one standard control (SC) and 3 modified chitosan groups (CSA1, CSA2 and CSA3). All the rats from group were fed a similar commercial diet.

Dose selection:
Toxicological study
Acute oral toxicity study of chitosan derivatives by Up and Down procedure. Acute oral toxicity study was carried out according to the OECD 425 guidelines. Five albino wistar rats weighing between 150-200 g were used. 2000 mg\kg compounds were given to the animals. These are observed individually at least once during the first 30 minutes after dosing, periodically during the first 24 hours (with special attention given during the first 4 hours), and daily thereafter, for a total of 14 days. Additionally observed for changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. As it does not observed any sign of toxicity so $1\backslash10^{th}$ dose of above dose as 200 mg\kg are taken as safe doses. (OECD 425)

Preparation of drug solution:
Compounds were dissolved in dilute acetic acid.

Volume of the injected drug solution:
The volume of drug solution was calculated based upon the body weight of the animal.

Route of administration
The calculated doses of compounds were administered orally. The poloxamer-407 was administered by intraperitoneal route.

Preparation of Poloxamer
The poloxamer was made at a final concentration of 500 mg/kg by dissolving the powder in distilled cool water; the solution was then kept refrigerated overnight to facilitate its dissolution. Needles and syringes used to administer poloxamer were cooled prior to administration to prevent poloxamer gelation within the syringe. Poloxamer was administered i.p to the rats.

Induction of Hyperlipidemia [14]
Hyperlipidemia was induced by Poloxamer (500mg/kg b. wt) administered i.p.

Experimental design
During the experimental period, body weight and food intake were recorded daily. All groups were fed the corresponding diets at the beginning of the experiment. The test drugs (SC, CSA1, CSA2, and CSA3) were administered orally for 12 days to the respective group of animals (Table 01). The hyperlipidemia was induced by single i.p. injection of poloxamer (500 mg/kg) 48 hr prior to blood collection. On 14th day the blood was collected by retro orbital sinus puncture under light ether anaesthesia. The blood was centrifuged at 3000 rpm for 10 minutes.

Table No. 01 Experimental design

Group	Initial 12 days	13th day
UC: Untreated control	Normal diet	Normal diet
PC: Polaxomer control	Normal diet	Normal diet + Polaxomer
SC: Standard control	Normal diet + SC	Normal diet + Polaxomer
CSA1	Normal diet + CSA1	Normal diet + Polaxomer
CSA2	Normal diet+ CSA2	Normal diet + Polaxomer
CSA3	Normal diet+ CSA3	Normal diet + Polaxomer

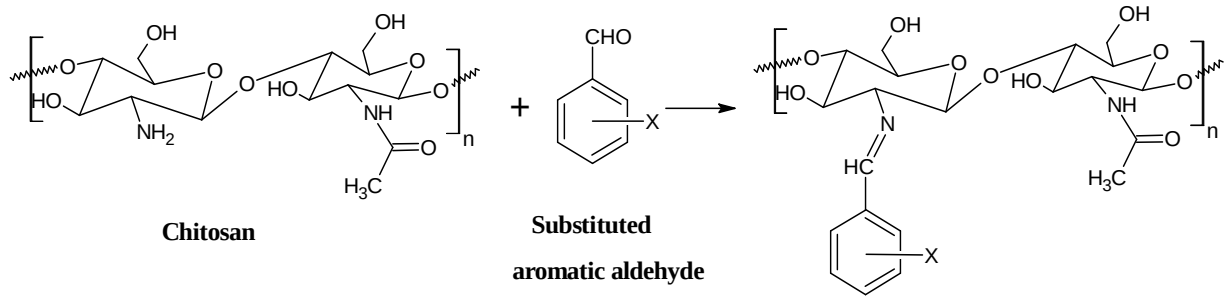
Estimation of Total cholesterol:
The estimation of total cholesterol from serum was done by using colorimetric method. Before the estimation the serum samples were kept frozen at -40^{0}C for 12 Hrs. For the estimation, Cholesterol kit (Patho lab, Kolhapur, India) and colorimeter were used. The readings of absorbance were recorded at 505 nm.

RESULTS AND DISCUSSION

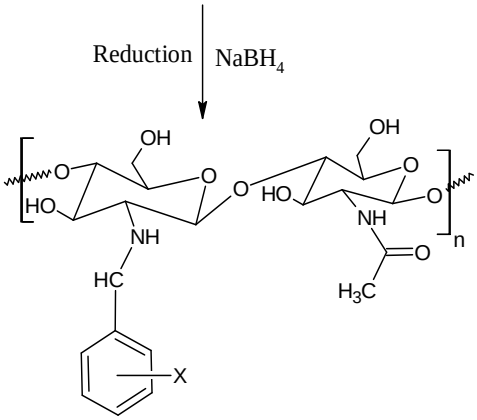
Synthesis of chitosan derivative by reductive N-arylation:
The derivative of chitosan with aromatic aldehyde was synthesized via the Schiff base intermediate. The reaction was carried by using homogeneous reaction between chitosan and aromatic aldehyde in methanolic acetic acid. Methanol was used to provide homogenous reaction medium towards uniform distribution of aldehyde towards chitosan repeating units. The representation of synthesis of N-aryl derivative (Scheme: 1).

Spectral confirmations of chitosan derivatives:
The spectral data of IR shows main bands of chitosan: OH stretching at 3444 cm $^{-1}$, N-H bending vibration at 1644 cm^{-1}, CH_3 symmetrical angular deformation 1384 cm^{-1}, C-N amino axial deformation at 1020 cm^{-1}. The IR spectra of N-aryl derivative of chitosan presented a strong absorption band at 1636 cm^{-1} attributed to the presence of secondary amines, which is not observed in chitosan.

NMR spectroscopic data base have shown variable peaks from aromatic functionality to the tetrahydropyran ring. Following are the ppm values for the same: The ppm value near 8 represents the aromatic functionality. Values at 3 are the representatives for the protons of secondary amine group whereas at 2.2 to 2.5 are the peaks for protons of hydroxyl functionality. Peaks from 5 to 7 contain the protons from tetrahydropyrans.



Chitosan **Substituted aromatic aldehyde** **Imine(schiff base) of chitosan**



N-aryl chitosan

X= $(OCH_3)_2$; $N(CH_3)_2$; C_6H_5 OH

Scheme 1: General scheme for the synthesis of N-aryl chitosan derivative

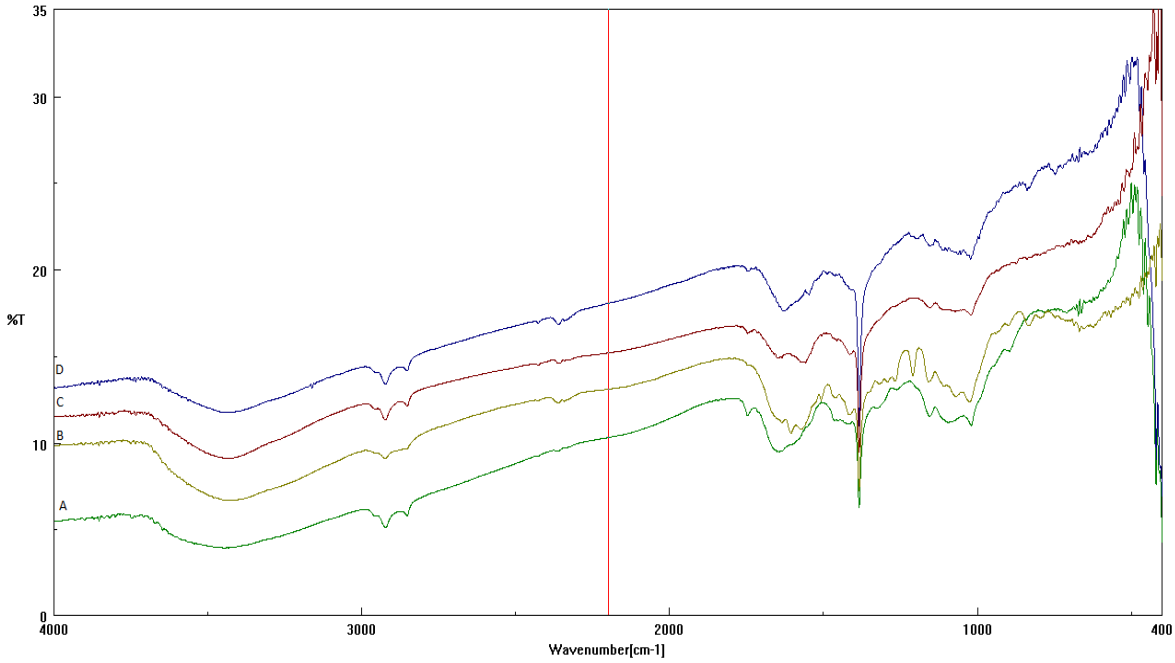


Fig.01: IR spectra of Chitosan and modified Chitosan derivatives

A: Chitosan; B: 2, 4-dimethoxybenzyl Chitosan; C: 2, 4-dimethylamino benzyl Chitosan;D: Hydroxy naphthyl chitosan

Hypocholesterolemic activity:

A. Body weight gain:

The body weight gain parameter of all the rats has been observed. The result shows the marked decrease in the change in body weight gain in the rats which are under treatment of chitosan as well as modified chitosan. There is

normal change in body weight gain was observed in the groups UC and PC. The change in body weight gain is shown in Table 02.

Table 02: Change in Body weight gain of animals.

Sr. No.	Group	Change in body weight gain (gm)			
		3 days	6 days	9 days	12 days
1	UC: Untreated control	14	24	33	44
2	PC: Polaxomer control	14	24	32	43
3	SC: Standard control	12	21	31	38
4	CSA1	12	19	28	35
5	CSA2	12	18	28	36
6	CSA3	12	19	27	34

B: Total serum cholesterol:

Total serum cholesterol was determined by using colorimetric method. Results shows the higher concentrations were found in Polaxomer induced rats. It may be due to its hyperlipidemic property. The group of rats which was induced by modified chitosan doses shows prominent results of hypolipidemia. The concentrations were found to be very low as compared with non-modified chitosan.

Table 03: Total cholesterol level

Sr. No.	Group	Total cholesterol (mg/dL)
1	UC: Untreated control	114.054 ± 11.16**
2	PC: Hyperlipidemic control(Poloxamer,500mg/kg i.p)	472.6832 ± 4.42**
3	SC	373.0864 ± 8.58**
4	CS-A1	312.8464 ± 41.272**
5	CS-A2	332.1233 ± 9.568**
6	CS-A3	338.5488 ± 23.498**

*All the values in Mean ± SD. **presents significant results.*

CONCLUSION

It was observed that the Chitosan when modified chemically by N-arylation, exhibits hypocholesteremic activity in rats. Unmodified Chitosan also shows the similar kind of activity but when modified these activities claims to be significantly good. Chemical modification allows Chitosan to employ ion-exchange property. It is believed to be one of the functions for delivering hypocholesterolemic properties.

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