NVEO
Natural Volatiles & Essential Oils

Engineered Modified Galactose, Pectin and Chitosan-Metoprolol Conjugates to Target Cardiovascular System

Smita Tukaram Kumbhar, *Shitalkumar Shivgonda Patil,* Manish Sudesh Bhatia

[1]*Department of Pharmaceutical Chemistry, DSTS Mandal's College of Pharmacy, Solapur, India. Email-smitakumbhar@gmail.com*

[2]*Department of Pharmaceutics, Ashokrao Mane College of Pharmacy, Peth Vadgaon, India. Email-shitalkumarpatil@yahoo.co.in*

[3]*Department of Pharmaceutical Chemistry, Bharati Vidyapeeth College of Pharmacy, Kolhapur, India. Email-drmsb1369@gmail.com*

Submitted: 11.02.2021; Accepted: 20.04.2021

Abstract

Targeted drug delivery to selective cell has emerged as one of the most significant areas of biomedical engineering research today. Therapeutic efficacy of a drug can be enhanced and optimized by localizing strictly it to a pathophysiologically relevant tissue system. The current study is aimed to develop the saccharide conjugates for targeted delivery of Metoprolol, a β-blocker. The selected saccharides viz. galactose (monosaccharide), pectin (polysaccharide), and chitosan (polysaccharide). The conjugates were engineered by grafting Metoprolol with the modified saccharides. The chemically modified saccharides conjugates were characterized by spectroscopic and thermal analysis. Drug release analysis and cellular uptake study was carried out using H9c2 cell lines. Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity. The results demonstrate that Metoprolol-modified saccharide conjugates can efficiently deliver the drug to the target. It can be concluded that the development of saccharide-drug conjugates can be an effective approach for the targeting of cardiovascular drug.

Keywords – Biopolymer, Chemical modification, Metoprolol, Targeting

I. INTRODUCTION

The ability to specifically target a drug to specific cells has the potential to significantly improve their therapeutic efficacy. Delivery of adequate doses of drug to specific sites promotes its therapeutic outcome wherever required and thus limits its side effects. Thus potentially results in a significant decrease of side effects[1, 2]. According to Martinez[3], the drug targeting concept is often associated with the use of carrier systems, which are potentially able to transport drugs, imaging agents or therapeutic genes selectively to the site of action.

Oligosaccharide and polysaccharide polymers obtained from natural origin are non-toxic, biocompatible and biodegradable. Additionally polysaccharides are more thermally stable than other biopolymers, like lipid and proteins[4, 5]. According to Sabyasachi[6], incorporation of the therapeutic agent into a chemically modified polymeric matrix, might protect the biologically active compound from degradation, improve absorption, control drug release, enhance the therapeutic efficacy, and so leads to the decrease in the frequency of administration. Chemical grafting is a process by which one or more species of blocks are

connected as a side chain to the main chain, resulting in the formation of macromolecular copolymers with altered physicochemical properties. Newly formed copolymer can be distinguished on the basis of number, length, and molecular structure of the side chains[7].

Cardiovascular diseases [17,18,19] like arrhythmia and hypertension are of major concern as they have hit large number of population across the world. Cardiovascular agents such as sympathetic antagonists show variety of untoward effects because of affinity of the agents towards the sympathetic receptors located in many organs. Hence it is important to target the drugs like β-blockers for their cardio-selective action. Targeted delivery of these drugs to cardiac tissues can be achieved by passive or active targeting[8].

Drug delivery to the cardiovascular system is different from delivery to other systems due to anatomical and physiological differences[9]. Cardiovascular cells are enriched with carbohydrate transporters like GLUT4 (Human Solute carrier family-2) which normally regulate the glucose transportation across the cardiac cell. The conjugations of cardiovascular drug with the oligosaccharides will be an attractive technique to manipulate the pharmacokinetic property of the drugs[10].

The aim of the current study is to modify the saccharides and develop saccharide conjugates for targeted delivery of Metoprolol, the β-blockers to increase the effectiveness of treatment [20,21] as well as decrease the adverse effects.

II. EXPERIMENTAL

2.1 Materials:

2.1.1 Chemicals: Atenolol (ATN) and Metoprolol (MET) were gifted by Wockhardt limited, Aurangabad. Galactose, pectin, and chitosan were provided by Loba Chemicals, Mumbai. Oxalic acid and thionyl chloride were supplied by Molychem, Mumbai. H9c2 rat heart cells of adherent nature were received from National Center for Cell Sciences (NCCS, Pune, India). Dulbecco's Modified Eagle's Medium (DMEM), L-glutamine, antibiotics (streptomycin-penicillin solution), fetal bovine serum (FBS), Trypsin-EDTA, Phosphate buffered saline solution (PBS), Hank's Balanced Salt Solution (HBSS), Tris-base and Triton-X 100 were purchased from Himedia lab, methanol (HPLC grade) was purchased from Qualigens. Silica gel aluminum plates $60F_{254}$ were purchased from Merck Pvt Ltd. HPLC column (4.6 mm×150 mm, 5µm ODS-3 and 100 Å) Prodigy™ C_{18} was purchased from Phenomenex. Tissue culture flask (75 cm^2) and flat bottom polystyrene 96 well-tissue culture plate were purchased from Tarsons Pvt. Ltd.

2.1.2 Instruments: Spectrophotometric analysis was carried out on a Systronics 2201 UV-Visible spectrophotometer with a spectral bandwidth of 2 nm and wavelength accuracy of ± 0.3 nm using a matched pair of 10-mm quartz cells. The FT-IR study was done on Nicolet-iS10 FTIR. Thermal behaviors were studied on Differential Scanning Calorimeter-Mettler-Toledo with a nitrogen flow rate of 40 ml/min and a heating rate of 10°C/min from 25 to 300°C. NMR spectrometer used for analysis of conjugates was carried out on a Bruker AV III 400 MHz. FTIR, DSC; 1H NMR and 13C NMR studies were carried out at the Central Instrumentation Facility, Punyashlok Ahilyadevi Holkar Solapur University,

Solapur, Maharashtra. HPLC analysis was carried out on Jasco MD-2010, multiwavelength detector, and equipped with Borwin® Version 1.5 software at Bharati Vidyapeeth College of Pharmacy, Kolhapur, Maharashtra. Cell line study was conducted at Biocyte Institute of Research and Development, Sangli, Maharashtra.

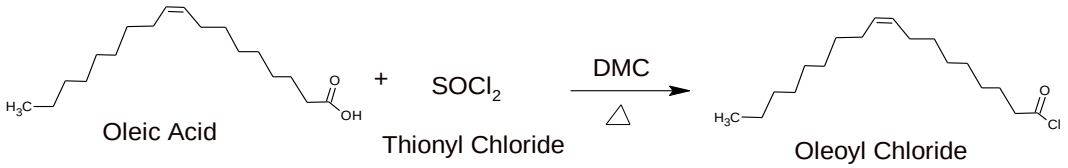
Synthesis of the Metoprolol conjugates by cross-linking the modified saccharides

The current work was aimed to chemically modify the selected saccharides and conjugate Metoprolol for cardiovascular targetting. A monosaccharide, galactose (G) and two polysaccharides *viz*. pectin (P) and chitosan (C) were chemically modified by using oleoyl chloride to synthesize esters *viz*. Galactose oleate (G1), Pectin oleate (P1) and Chitosan oleate (C1) respectively. The chemical modification of saccharides followed by conjugation of Metoprolol with the modified saccharide was carried out in a three-step procedure. Conjugates were coded as MG1, MP1 and MC1 respectively.

Step I: Synthesis of oleoyl chloride:

The synthesis of oleoyl chloride was done from oleic acid as per the procedure described by Menalda[11] which involves treatment of the acid with thionyl chloride in an inert organic solvent.

2 ml of oleic acid and 16 ml of dichloromethane were taken and stirred in a round bottom flask. The resultant solution was heated to reflux (40-45°C) for about 1 h. 6 ml of thionyl chloride solution was gradually added to the refluxing solution for about 30 min and stirred for 3 h. A brown colored acid chloride solution was formed, which was cooled to 15-25°C and used in the subsequent reaction.

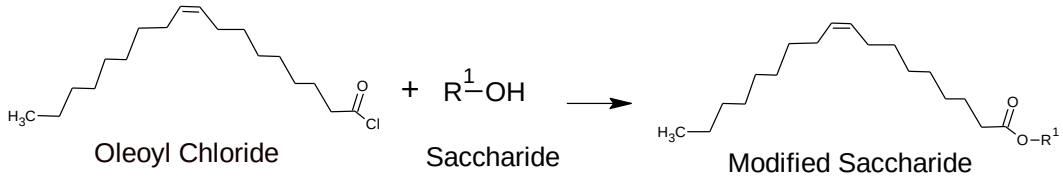


Scheme 1: Synthesis of oleoyl chloride

Step II: Modification of saccharides using oleoyl chlorides:

The Schotten-Baumann reaction[12] was followed for the modification of the Saccharide.

10 ml of 20% w/v acid chloride in ethanol was gradually added under stirring over a period of 2 h to ethanolic solution of saccharides. Reaction product was collected, washed and dried in hot air oven at 37ºC.

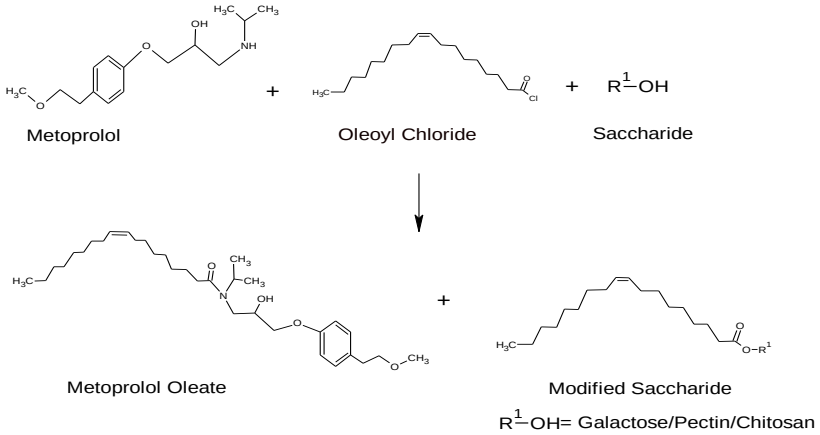


R^1–OH= Galactose/Pectin/Chitosan

Scheme 2: Modification of saccharides using oleoyl chlorides

Step III: Synthesis of drug-modified saccharide conjugates:

Synthesis of drug-saccharide conjugates was developed by method reported by earlier with slight modification[13]. 10 ml of 20% w/v acid chloride in ethanol was gradually added under stirring over a period of 2 h to ethanolic solution of 2 g of saccharide and 1 g of drug. Reaction product was collected, washed and dried in hot air oven at 37ºC.



Metoprolol-Modified Saccharide Complex and Conjugates

Scheme 3: Synthesis of MET-MS conjugates

2.3 Physicochemical characterization of modified saccharides:

Characterization of chemically modified saccharides was done by determining melting point, partition coefficient, swelling factor and ester value. The confirmation of modification reaction was done by TLC, FTIR, and DSC study.

2.4 Characterization of Metoprolol-modified saccharide conjugates:

The synthesized conjugates were characterized for various physicochemical parameters. The confirmation of the reaction was done by melting point and TLC as primary parameters followed by FTIR, NMR and DSC analysis on instruments as specified in the Materials section.

2.4.1 Drug Release Analysis:

H9c2 Cells Preparation:

H9c2 rat heart cells of adherent nature were maintained in DMEM with 20 µM L-glutamine, 0.45% glucose and 10% v/v heat inactivated FBS. Gentamicin sulfate (50 µg/ml), Penicillin (100 IU/ml), Streptomycin (10 µg/ml) and amphotericin B (25 ng/ml) were added to prevent microbial contamination during maintenance in a humidified CO_2 incubator (New Brunswick, Eppendorf). Cells in a confluent layer were rinsed cells with 10 ml PBS (pH 7.4) to remove any residual medium. 0.25% w/v Trypsin-EDTA was added to dissociate the cells from confluency to obtain homogenous suspension. The cells were mixed with 0.4% w/v Trypan blue solution in a ratio of 1:1 and counted by hemocytometer to obtain 10^4 cells.

Cellular uptake study:

For the cellular uptake study[14], the above H9c2 cells were cultured and incubated in humidified incubator so as to reach 2×10^5 cells. After incubation, the cells were washed with 200µL HBSS and incubated for equilibrium. Metoprolol-saccharide conjugates (50 µg/ml), positive control- Metoprolol

(100 µg/ml) and negative control- sterile water were added in each well, incubated for 12 h. Subsequently, the cells were removed, washed with ice-cold PBS (pH 7.4), treated with 0.5%v/v Triton-X 100 and incubated for 30 min. For the preparation of samples for HPLC analysis, methanol: water (90:10) mobile phase was added in cells, and centrifuged (REMI, Mumbai, India). After centrifugation, the supernatant layer was necessarily diluted and spiked for HPLC analysis to study cellular uptake in H9c2 rat heart cells.

Chromatographic conditions:

RP-HPLC method was adopted for estimation of metoprolol in H9c2 rat heart cells. HPLC system (Jasco MD-2010, multiwavelength detector) with Borwin Version 1.5 software, Prodigy™ C_{18} column as stationary phase and methanol: water (90:10) as mobile phase was used for chromatographic analysis and data acquisition. Flow rate was adjusted as 1 ml/min and 20 µl samples were injected. The intensity of peak was measured at 223 nm.

2.4.2 Toxicity analysis of conjugates:

Brine shrimp lethality bioassay was carried out to investigation of the cytotoxicity of synthesized conjugates. Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs, in sterile artificial seawater (prepared using sea salt 38 g/L and adjusted to pH 8.5 using 1N NaOH) under constant aeration for 48 h in a 1L conical vessel. After hatching, active nauplii free from egg shells were collected from brighter portion of the hatching chamber and used for the assay. Ten nauplii were drawn through a glass capillary and placed in each vial containing 4.5 ml of brine solution. A group of nauplii was kept untreated as a control group. In test group, 0.5 ml of test samples in different concentrations (10, 50, 150 µg/ml) was added to 4.5 ml of brine solution and maintained at room temperature for 24 h under light and surviving larvae were counted[15,16].The experimental model selected for toxicity studies does not require the approval of institutional animal ethical committee.

Determination of Lethal dose:

The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. LC_{50} values were obtained from the best-fit line plotted concentration verses percentage lethality. The data were analyzed with a Finney computer programme (Probit analysis) to determine LC_{50} values.

III. RESULTS AND DISCUSSION

Modification of saccharides using acid chloride was carried out by the method reported by Schotten Baumann reaction[31]. Thus three different modified sachharides *viz*. galactose oleate, pectin oleate and chitosan oleate were synthesized.

3.1 Characterization of Modified Saccharides:

Characteristics of modified saccharides are depicted in Table 1. It can be observed that there is decrease in the melting points of the modified saccharide compared to the respective saccharide indicating the completion of esterification reaction which is clearly evident from the ester values. Pectin and chitosan demonstrate significantly greater ester values compared to that of monosaccharide galactose. Modified Saccharides show comparatively higher partition coefficients than that of respective saccharides. This result is supported by solubility study in water and organic solvent, which indicate decreased

hydrophilicity and increased lipophilicity. Swellability of modified saccharides were decreased in comparison with the parent compound which may be due to decreased hydrophilic interactions. Thus it appears that the modified saccharides may possess the ability to delay the drug dissolution in aqueous medium. Thus these may be potential auxiliary agents which can be used in extended release pharmaceutical dosage forms.

Table 1: Characteristics of synthesized Modified Saccharides

Code	% Yield	Melting point (°C)	Partition Coefficient	Swelling % ± SD	Ester value	R_f
G	-	178-180	1.28	124±0.44	-	0.2
G1	78.21	153-155	4.74	114±4.97	61.71	0.63
P	-	166-168	2.2	420±3.23	-	0.14
P1	92.60	146-148	6.45	316±0.84	282.97	0.48
C	-	89-91	1.95	204±2.29	-	0.25
C1	72.33	73-78	5.88	186±4.65	206.57	0.55

3.2 Characterization of Metoprolol-Modified Saccharide Conjugates:

The synthesized conjugates were characterized for various physicochemical parameters including percentage yield, melting point and R_f value by thin layer chromatography followed by FTIR, DSC, ^{1}H NMR and ^{13}C NMR analysis on instruments as per specified in the Materials section. Confirmation of synthesized compounds was done by the results of the analysis.

Conjugate MG1: Color: colorless, yield: 88%, m.p.: 168-170°C, R_f: 0.47, IR: (aldehyde) 1737 cm^{-1}, NH (amide) 3195 cm^{-1}, NH (amine) 3378 cm^{-1}, OH at 3190 cm^{-1}, DSC: Tg: 163°C, NMR: ^{1}H NMR (400 MHZ; DMSO): 0.8 ppm (t)-Terminal CH_3, 1.1 to 1.3 ppm (m)- CH_3 of isopropyl and CH_2 of oleyl side chain, 1.55 ppm (bs)- CH_2, 1.98 ppm (bs) - CH_2, 2.1 ppm (t)- αCH_2 of oleyl side chain, 2.7 ppm (t)–Benzyl CH_2, 3.2 to 4.5 ppm (m)- $OCH_3/OCH_2/OCH/OH/NCH_2$ protons, 5.0 ppm (s)-Anomeric CH proton, 5.2-5.4 ppm (m)- alkene CH of oleyl side chain, 6.0 ppm (bs)-hydroxyl proton of sugar, 6.78 ppm (d)- aromatic protons, 7.07 ppm (d)- aromatic protons, ^{13}C NMR (100 MHZ; DMSO): δ 156.77 ppm, δ 131.79- 112.47 ppm, δ 102.53-61.13 ppm, δ 58.32 ppm, δ 29.53-14.29 ppm.

Conjugate MP1: Color: pale yellow, yield: 80%, m.p.: 68-72°C, R_f: 0.34, IR: aldehyde 1738 cm^{-1}, NH (amide) 3197 cm^{-1}, NH (amine) 3381 cm^{-1}, (OH) 2923 cm^{-1}, DSC: Tg 158°C. NMR: ^{1}H NMR (400 MHZ; DMSO): 0.8 ppm (t)-Terminal CH_3 of oleyl side chain, 1.1 to 1.3 ppm (m)- CH_3 of isopropyl and CH_2 of oleyl side chain, 1.55 ppm (bs)- CH_2 of oleyl side chain, 1.98 ppm (t) - CH_2 of oleyl side chain, 2.1 ppm (t)- αCH_2 of oleyl side chain, 3.2 to 4.3ppm(m)- $OCH_3/OCH_2/OCH/OH/NCH_2$ protons, 5.2-5.4 ppm (m)- alkene CH of oleyl side chain, 6.78 ppm (d)- aromatic protons, 7.07 ppm (d)- aromatic protons, ^{13}C NMR (100 MHZ; DMSO): δ 170.92 ppm, δ 134.62-128.49 ppm, δ 107.76 -102.73 ppm, δ 98.33-61.64 ppm, δ 159.59 ppm, 59.96 ppm, δ 33.93-14.29 ppm.

Conjugate MC1: Color: pale yellow, yield: 80%, m.p.: 70-75°C, R_f: 0.48, IR: aldehyde 1560 cm^{-1}, NH (amide) 3170 cm^{-1}, NH (amine) 3383 cm^{-1}, OH 3135 cm^{-1}, DSC: Tg 74°C, NMR: ^{1}H NMR (400 MHZ; DMSO) 0.8 ppm (t)-Terminal CH_3 of oleyl side chain, 1.1 to 1.3 ppm (m)- CH_3 of isopropyl and CH_2 of oleyl side chain, 1.55 ppm (bs)- CH_2 of oleyl side chain, 2.3 ppm (t)- αCH_2 of oleyl side chain, 3.2 to

4.3ppm (m) -OCH_3/OCH_2/OCH/OH/NCH_2 protons, 6.8 ppm (d)- aromatic protons, 7.1 ppm (d)- aromatic protons, ^{13}C NMR (100 MHZ; DMSO): δ 169.65 ppm, δ 161.64 ppm, δ 140.85-134.46 ppm, δ 130.85-102.10 ppm, δ 99.29-63.15 ppm, δ 51.52 ppm, δ 29.53.

3.3.1 Toxicity assay:

Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of synthesized Metoprolol conjugate. The percentage lethality was determined by comparing the mean surviving larvae of the test and control tubes. LC50 values were obtained from the best-fit line plotted concentration verses percentage lethality. All three Metoprolol conjugate did not show any significant toxicity demonstrating much higher levels of LC_{50} indicative of a good safety profile. Results of toxicity are summarized in Table 2.

Table 2: Results of toxicity study

Drugs	Conc. of compound µg/ml	Total no. shrimps used/tube	Shrimp survived			Total No. of Shrimp survived	Percentage mortality	LC_{50} (µg)	(95% confidence interval)
			T1	T2	T3				
MG1	50	10	9	8	8	26	10.34	231.53 ± 7.52	212.15 -249.53
	100		9	8	8	25	13.79		
	150		7	7	7	20	31.03		
MP1	50	10	8	8	9	25	13.79	231.58 ± 10.01	207.60 -257.45
	100		7	8	8	23	20.68		
	150		6	6	7	19	34.48		
MC1	50	10	8	8	9	25	13.79	231.57 ± 10.51	206.07 -258.31
	100		7	8	8	23	20.68		
	150		6	6	7	19	34.48		

3.3.2 Cellular uptake study:

RP-HPLC analysis was conducted to estimate the concentration of Metoprolol in Metoprolol-saccharide conjugates across H9c2 rat heart cells. The intensity of peak measured at specific retention time is a measure of concentration of metoprolol present in the cells. Metoprolol and its saccharide conjugates were added in the cells, incubated, washed and treated carefully with cold PBS to remove metoprolol from the adhered cells. Further, chromatographic methods ensured the analysis of drug from chemically disrupted cells.

In view of results in Figure 1, metoprolol concentration in H9c2 rat heart cells was observed to contain 25 to 30 µg/ ml after loaded with 50 µg/ ml of metoprolol and metoprolol saccharide conjugates. The reason behind the accumulation of drug in cells was supposed to bind drug saccharide conjugates at receptors as evidenced by docking analysis. Further, improvement in intracellular drug transport was

also claimed due to bypassing drug transport system so as to favor dynamic balance between intra and extracellular concentration of drug during extended period of incubation. Figure 2 shows the percentage of drug uptake in H9c2 cells after 12 h incubation was showed in the range of 50% to 59%. The chemical conjugation of metoprolol and various saccharide units influenced drug permeation across cells and ensured drug stability across cells. The individual HPLC chromatograms of metoprolol and metoprolol-saccharide conjugates are after treatment of H9c2 rat heart cells are mentioned in Fig. 3.

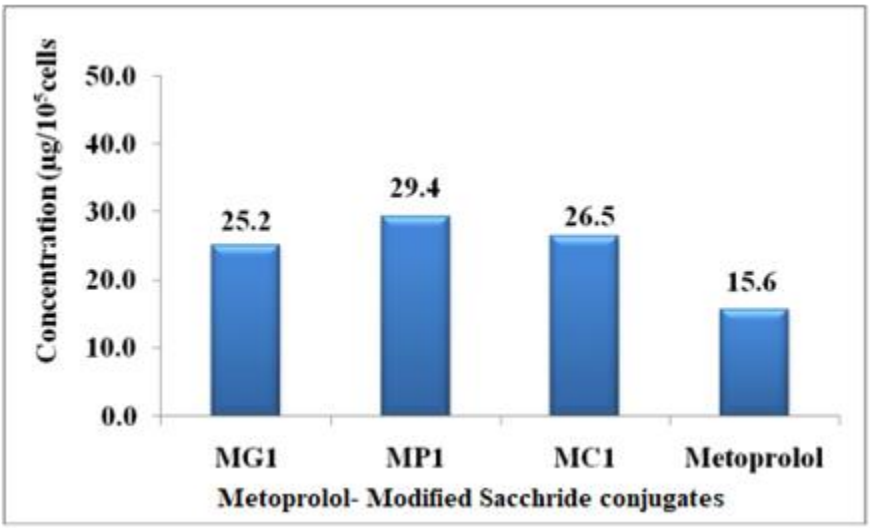


Figure 3: Metoprolol concentration in Metoprolol- Modified saccharide conjugates in H9c2 rat heart cells

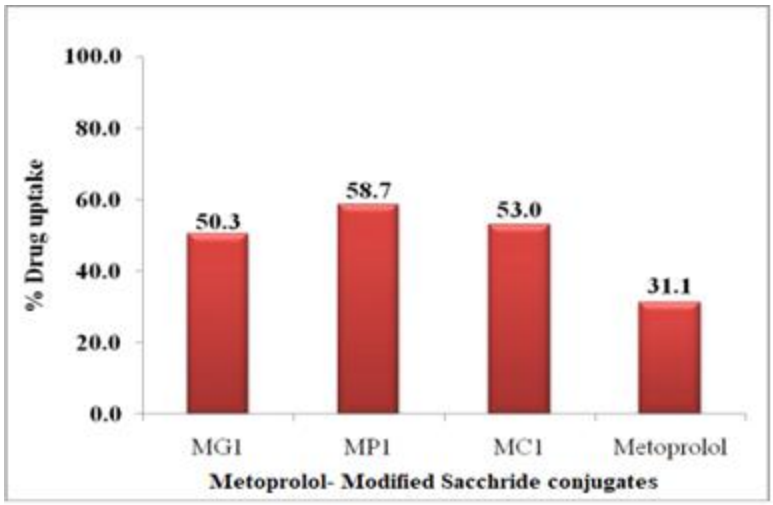


Figure 4: Percentage of Metoprolol and Metoprolol- Modified Saccharide conjugates uptake across H9c2 rat heart cells

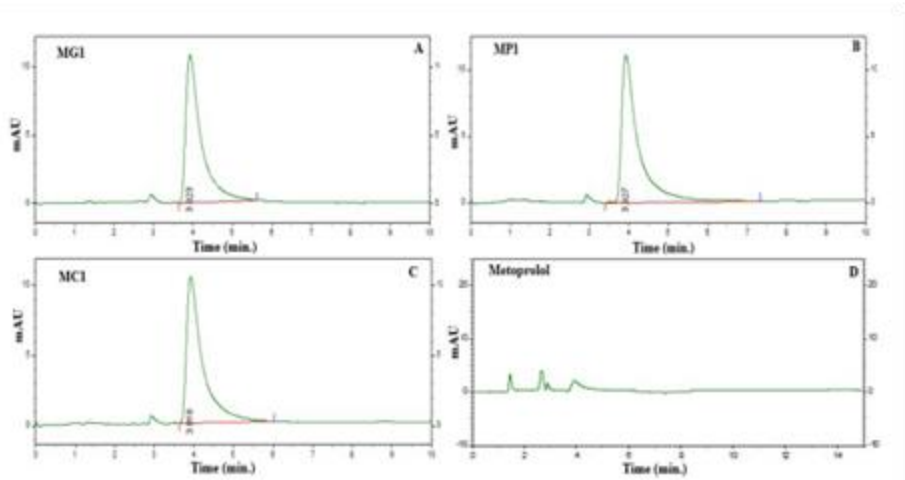


Figure 5: HPLC chromatogram of Metoprolol- Modified Saccharide conjugates A) MG1, B) MP1, C) MC1 and D) Metoprolol in H9c2 rat heart cells

IV.CONCLUSION

Different chemically modified saccharides were successfully conjugated to the Metoprolol in order to improve the availability of the drug to the cardiac cells for better control of cardiovascular disorders as well as reduction in the side effects. The modified saccharides show increased lipophilicity, interestingly also improving the swelling characteristics. This finding creates an opportunity to use the modified saccharides as excipient in extended drug delivery formulations. The chemically modified saccharides with cardiovascular drug were characterized by FTIR and DSC. The synthesized conjugates are found to be stable. Results of cell line studies prove that the drug can be successfully targeted for selective cardiac delivery. The toxicity study provides clear evidence of the safety of the prepared compounds. Thus, this approach to enhance delivery and therapeutic outcomes of Metoprolol by conjugating with biodegradable and biocompatible polymeric system is promising to improve selectivity of drug delivery for treatment of cardiovascular diseases.

REFERENCES

Hyo Kyung H., “Targeted prodrug design to optimize drug delivery”, AAPS Pharmscitech, vol. 2(1), pp. 6, 2000.

Namazi, H., Fathi, F. and Heydari, A., “Nanoparticles based on modified polysaccharides” The Delivery of Nanoparticles. In Tech Publisher, pp.149-184, 2012.

Martinez, A., Fernandez, A., Perez, E., Benito, M., Teijon, J.M. and Blanco, M.D., “Polysaccharidebased nanoparticles for controlled release formulations”, The Delivery of Nanoparticles, In Tech Publisher, pp. 185-222, 2012.

Benjamin, G.D., Mark, A.R., “Drug delivery systems based on sugar-macromolecule conjugates”, *Current Opinion in Drug Discovery & Development*, vol. 5(2), pp. 279-288, 2002.

Baptiste, T., Kai-Cheng, Y., Xi-Le, H., Marion, M., Guo-Rong, C., and Sebastien, V., “Fluroscent glycoconjugates and their application”, *Chemical Society Reviews*, vol. 49, pp. 593-641, 2020.

Sabyasachi, M., Somdipta, R., Biswanath, S., "Polysaccharidebased graft copolymers in controlled drug delivery", *International Journal of PharmTech Research*, vol. 2(2), pp. 1350-1358, 2010.

Pathania, D., Sharma, R. and Kalia, S., "Graft copolymerization of acrylic acid onto gelatinized patato starch for removal of metal ions and organic dyes from aqueous system", *Advanced Materials Letters*, vol. 3(2), pp. 259-264, 2012.

S., Hiroyuki, O., Hisakazu, M., "Gold nanoparticles conjugated with glycopeptides for lectin detection and imaging on cell surface", *Protein Peptide Letters*, vol. 25(1), pp. 84-89, 2018.

Fei D, Yongmin Z., "Biomedical applications of Glycoconjugates", *Mini-Review in Medicinal Chemistry*, vol. 18(18), pp. 1508-1523, 2018.

Dvir, T., Bauer, M., Schroeder, A., Jonathan, H.T., Anderson, D.G., Langer, R. "Nanoparticles targeting the infarcted heart", *Nano Letters*, vol. 11(10), pp. 4411-4414, 2012.

Menalda, F., "Quantitative investigation of the Schotten-Baumann reaction", *Recueil Des Travaux Chimiques des Pays-Bas*, vol. 49(10), pp. 967-995, 2010.

Vogel, Arthur, Furniss, B.S., Hannaford, A.J., Smith, P.G. and Tatchell, A.R, "Vogel's textbook of practical organic chemistry", 5th ed., Person Publications, pp 692, 1996.

Bhatia M., Deshmukh R., and Bhatia N., "Chemical Modification of Pectin, Characterization and Evaluation for Drug Delivery", *Scientia Pharmceutica*, vol. 76, pp.775-84, 2008.

Zordoky, B., El- Kadi, A., "H9c2 cell line is a valuable in vitro model to study the drug metabolizing enzymes in the heart", *Journal of Pharmacological and Toxocological Methods*, vol. 56, 3, pp. 317-322, 2007.

Berghot, M. A., Kandeel, E. M., Abdel-Rahman, A. H. and Abdel-Motaal, M., "Synthesis, antioxidant and cytotoxic activities of novel naphthoquinonederivatives from 2,3-dihydro-2,3-epoxy-1,4-naphthoquinone", *Medicinal Chemistry*, vol. 4, pp. 381-388, 2014.

Riser M. J., Gu Z. M., Fang X. P., Zeng L., Wood K. V. and J. L. Mclaughlin., "Five novel mono-tetrahydrofuran ring acetogenins from the seeds of Annona muricata", *Journal of Natural Product*, vol. 59(2), pp. 100-108, 1996.

Sharma, P., Saxena, K., & Sharma, R.. Heart disease prediction system evaluation using C4.5 rules and partial tree doi:10.1007/978-81-322-2731-1_26. , 2016.

Saxena, R., Johri, A., Deep, V., & Sharma, P.. Heart diseases prediction system using CHC-TSS evolutionary, KNN, and decision tree classification algorithm doi:10.1007/978-981-13-1498-8_71, 2019.

Aggarwal, A., Alshehri, M., Kumar, M., Alfarraj, O., Sharma, P., & Pardasani, K. R. Landslide data analysis using various time-series forecasting models. Computers and Electrical Engineering, 88 doi:10.1016/j.compeleceng.2020.106858.

Sharma, P., Alshehri, M., Sharma, R., & Alfarraj, O. (2021). Self-management of low back pain using neural network. Computers, Materials and Continua, 66(1), 885-901. doi:10.32604/cmc.2020.012251.

Sharma, P., Alshehri, M., Sharma, R., & Alfarraj, O. (2021). Self-management of low back pain using neural network. Computers, Materials and Continua, 66(1), 885-901. doi:10.32604/cmc.2020.012251.