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A retrospective review on importance and various preparation of low molecular weight heparin for cardio vascular diseases

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ABSTRACT

Low-molecular-weight heparin (LMWH) are most importantly used in the clinical practice for cardio vascular diseases from 1990. The LMWH was a sulfated polysaccharide obtained from animal sources and some in natural especially from marine sources. The actual anticoagulant has the molecular weight about 25000 Daltons which have bleeding as side effect in the cardiovascular diseases. The LMWH are prepared by enzymatic or chemical hydrolysis the length of the heparin chain is reduced and also in same manner the molecular weight also reduced below 10000 Daltons. So, it has main advantage to reduce the bleeding in the cardiovascular diseases. The LMWH eliminated through the renal and it was not given to the patients with renal dysfunction. The LMWH of some product are still in clinical trials in order to reduce its side effects. The commercial LMWH preparation has concentrated in animal sources by killing them and lungs, intestine etc are used to prepare heparin. Here the alternate sources are discussed in order from killing the animal. This review summarizes the importance, difference between commercial heparin and LMWH, mechanism, preparation, LMWH products, clinical trials and LMWH from marine sources, Enzymatic degradation in shrimp species, Preparation of LMW heparin by chemically modified Fractions, Chromatography separation of LMW Heparin are discussed in this review.

1. Introduction

Heparin is a naturally-occurring polysaccharide used as anticoagulation when there is coagulation present. The heparin which is prepared by animal sources varies its molecular weights chain varying size from 5000 to 40000 Daltons and this is used as pharmaceutical grade heparin. Heparin prepared from natural source can be given to prevent thrombosis. Therefore, the difference between natural LMW heparin and unfractionated heparin effect can be difficult to predict. The molecular weight of LMW Heparin varies from 4000 to 10000 Daltons. Closely monitor the coagulation parameters when unfractionated heparin is given to patients. The LMW Heparin was prepared by fractionation and depolymerization process it consist of short chain and defined as heparin salts having the molecular weight of average of 8000 Daltons and which 60% of the chain is less when compared to unfractionated heparin, it has potency of 70 units/mg of anti-factor Xa activity to its anti- thrombin ratio is > 1.5. Low molecular weight heparins (LMWHs) are used widely in the VTE (Venous Thromboembolism). VTE comprises thrombosis in deep vein and pulmonary embolism (PE), a complication of DVT where some or all of the thrombus breaks away and lodges in the pulmonary arteries. It is difficult to get a true picture of incidence of VTE. Currently, it is estimated that one person per 1,000 will be affected by thrombosis (DVT).[1] The anticoagulant that occurs

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naturally is heparin and Medicinal heparin is usually derived from an animal source, usually mucosal tissue from pigs. LMWHs are manufactured by the enzymatic or chemical depolymerization of unfractionated heparin (UFH).[2] UFH binds to anti-thrombin, activating it and leading to inhibition of thrombin and factor Xa. LMWHs exert their effect on factor Xa Due to chain short length rather than inhibiting thrombin. Heparins and other anticoagulants do not dissolve blood clots; they prevent clots forming and prevent clots getting larger if already formed but it is the body itself that destroys the thrombus.

1.1. LMWH differ from uh in structure and mechanism of action

Unfractionated heparin was a mixture of polysaccharide chains with average molecular weight is 20000 Daltons. LMWH are derived from UH with enzymatic are chemical de-polymerization. The average molecular weight was 5000 Daltons. The major aim of the coagulation to create thrombin, factor Xa which is responsible for the clotting of blood by conversion of fibrinogen to fibrin clot. From pro-thrombin the thrombin was generated by activated action factor X. The UF and LMW Heparin ultimate aim is to act as anticoagulant by activating the antithrombin (previously known as antithrombin III) Once heparin react with antithrombin this activate the inactivating coagulation enzymes thrombin (factor Ha) and factor Xa to avoid the coagulation. The mechanism of action between the UH and LMWH is only the inhibition of factor Xa and thrombin. By conformational change in size of heparin chain stop the action of factor Xa by simply binding to anti thrombin. The heparin molecule chain should be long to bind with anti-thrombin and thrombin to inactivate the thrombin to prevent the clot and form ternary complex. The UFH has long chain as ternary complex and in case of LMW Heparin has the half the chain of UFH in order to produce the anticoagulant effects. Therefore, unlike UH, which has equivalent against factor Xa activity and thrombin LMWHs have greater activity against factor Xa.[3]

1.2. Importance of LMW heparin

In unstable angina the aspirin and heparin are standard therapy. However, LMW Heparin have more advantage than unfractionated heparin in case of predictable effects in anticoagulant. In LMW Heparin no need to monitor for anticoagulation, less incidence of thrombocytopenia and activated platelets of inhibited resistance. About 10 countries with 176 centre the Investigators used subcutaneous enoxaparin (low-molecular-weight heparin) with I.V UH in unstable angina patients or non-Q-wave myocardial infarction. The study was conducted in double blind trial with 3,171 patients, the history was having chest pain for 10mins with ischemic diseases, the 1mg of subcutaneous LMW Heparin per body weight was given for every 12 hrs in order to achieve the therapeutic level of APTT (activated partial thromboplastin). Within 12 to 24 hrs only 46% of the patients achieved an APTT for 55 to 85 sec in unfractionated heparin. The incidence of death in case of MI or angina by 14 days was significantly low in the LMW Heparin group (16.6% vs 19.8%, odd ratio,0.80). The OR are in reduce in LMW Heparin and notable for MI (OR 0.70) Angina (OR 0.80). At 30 days the LMW Heparin persisted the advantage over unfractionated heparin. The risk of haemorrhage[4] between the groups no significance in the difference. The LMW Heparin clinical advantages are predictability, long half-life, less bleeding, dose- dependent levels are good for the given anti thrombotic effects. Furthermore, the use of LMW Heparin in short term the patients associate with thrombocytopenia which is mediated by immune response and the use of unfractionated heparin which lower the osteoporosis when compared to standard. The waiting for oral anticoagulant by treating with thrombosis and the LMWH is used in this case to administered once or twice daily according to their body weight when the patients are high risk in DVT. The APTT need not be monitored and also the dosage was not adjusted. So, the use of LMW Heparin is given S.C. As an outpatient with or without help of nursing assistance who is visiting the home or family member which is possible in cost effective is also less.

1.3. Preparation of LMW heparin using porcine intestine

Heparin According to Roger Debri[5] procedure two batches of LMW Heparin were prepared using porcine intestine heparin. Briefly to the benzethonium chloride to form quaternary salt the heparin was added in it and dried and collect the salt precipitated. The final resulting dry powder was redissolved with dichloromethane and incubated at 400 c for 12 hrs by adding benzyl chloride to the powder and heparin benzyl ester was formed by centrifugation and formed one is collected. From the collected one the depolymerization process was performed by incubating 1g of heparin benzyl ester with 25ml of 4g/L sodium hydroxide solution. At 550c the reaction temperature is controlled for 2hrs in a water bath after the reaction is completed the hydrochloric acid with sodium hydroxide is used for neutralization of excess one. The final LMW Heparin product was precipitated with methanol for dialyzed membrane of molecule weight cut off 1 kDa and lyophilized.

1.4. Preparation of LMW heparin using bovine lung

Using bovine lung, a total of five batch LMW Heparin was prepared as the starting material. First batch is prepared using the same process as porcine intestine

procedure which is given above. The batch two was prepared by as modified processing conditions which is alkaline was reduced to 3.5g/L and the temperature was decreased to 500c in the depolymerization step. The next three batch was prepared by using the final processing conditions. The amount of alkaline, the reaction time and the depolymerization temperature were adjusted to 3.5g/L 500c and 6hrs respectively.

1.5. LMW Heparin prepared by chemical and enzymatic depolymerization

By electro focusing it shows that commercial heparin preparations are composed of molecules with different molecular masses ranging from 3 to 30 kDa. In large scale with different fraction are prepared and with high molecule weight exhibit a potent anticoagulant in vitro the values ranging from 200 to 300 IU/mg and molecular weight was 3kDa or less showed a negligible anticoagulant effect about 10IU/mg. This molecule is assayed in an in vivo model for thrombosis. The UF and LMW Heparin found to be antithrombotic drugs. The clinical results are based upon the in-vivo animal model for thrombosis with different heparin preparation this could be account in case of clinical trials. Since the antithrombotic activity are high in the preparation containing the LMW Heparin with in vivo and only 5% are accountable in commercial heparin. So, heparin is depolymerized, the depolymerization are obtained by Fento reaction cleaves of heparin molecules in moieties of glucuronic acid and by degradation with heparinase II.[6] Using this enzyme the it was used to produce more yield of LMW Heparin as shown by the electro focusing profile of both standard and LMW Heparin. When this LMW Heparin are tested in vivo in animal studies in venous thrombosis model in the mesenteric vein the ligature of the vena cava with kaolin is injected and into femoral vein it shows more potent when compared with standard heparin.

In thrombin inhibition the LMW Heparin have less anticoagulant effect in whole plasma assay and exhibit more anti-factor activity Xa and also reduce the thrombin formation in vivo. The UFH and LMW Heparin have thrombin as binding site for antithrombin activity and factor Xa another one. The interacted LMWH with factor Xa which shows low anticoagulant activity and in vitro as high anti Xa activity. The heparin can modulate in both sites. This could able to find led in pharmaceutical industries to produce or search in different methods to prepare the LMW Heparin. The Fenton reaction are famous for preparation of commercial heparin with nitrous acid degradation esterification and ß- elimination (enoxaparin), heparinase (tinzaparin), and molecular sieving. In this method preparation there is structural difference in LMWH as commercially and food and drug administration consequence as different type of drugs. The three different groups of LMWH available in the market are enoxaparin, dalteparin and nadroparin. There is increase in sales of LMW Heparin in last decade which is reaching to 10 billion dollars in 2018 compared to 150 million dollars of standard heparins. The LMW Heparin are examples to use as a reviewed.

1.6. Low molecular weight heparin products

In LMW Heparin various method are used for the manufacturing namely by depolymerizations and also by oxidative depolymerizations with hydrogen peroxide the product is listed below namely depolymerisation of oxidative with hydrogen peroxide, isoamyl nitrate cleavage with deaminative, benzyl ester of heparin cleavage of alkaline beta- elimination, with cu2+ and hydrogen peroxide depolymerization, heparinase enzyme with beta-elimination cleavage, nitrous acid cleavage are the various LMWH preparation used in current market to develop the product are shown in Table 1.

Table 1: Comparison of low-molecular-weight heparin preparations

Preparation	Method of Preparation	Mean Molecular Weight	Anti-xa,Anti-iia ratio
Ardeparin (Normiflo)	Peroxidative depolymerization	6000	1.9
Dalteparin (Fragmin)	Nitrous acid Depolymerization	6000	2.7
Enoxaparin (Lovenox)	Benzylation and alkaline depolymerization	4200	3.8
Nadroparin (Fraxiparine)	Nitrous acid depolymerization	4500	3.6
Reviparin (Clivarine)	Nitrous acid depolymerization, chromatographic purification	4000	3.5
Tinzaparin (Innohep)	Heparinase digestion	4500	1.9

2. Clinical Trials Comparing of LMW Heparin

The acute myocardial infarction risk was reduced with first small open trial comparing with LMW Heparin and aspirin with UFH and aspirin or aspirin alone. Based upon these three large studies was done in unstable angina patients. Double blind was first done with large randomized[7] trial with 1506 patients in unstable angina as control placebo studies or FRISC study by Q-wave myocardial infraction patients. The LMW Heparin was given to experimental group at 120U/Kg and 7500 anti-Xa units of LMWH for six days twice daily for 35 to 45 days. The placebo was given to the control group, all patients receive aspirin. The LMW Heparin shows decrease in 60% of myocardial infraction death by six days and in patients of 741 received LMWH

shows death of 1.8% and develop myocardial infarction with 4.7% of patients with placebo received. In case of revascularization, composite near to the dead points shows difference in significance of decrease in disease at fourty days of the myocardial infarction and rate of death are composite end point persisted. A change has happened by replacing the low dose maintaining after the high dose given to the patients with LMWH. The dose is 7500 anti-Xa units of LMWH once daily. At 6 days of high dose LMWH it produces inadequate protection during low dose maintained. No longer evident of two groups for four to five months follow up the significant difference in myocardial infraction death rates or revascularization. The 15.3% and 14% (P=0.41) are death rate in control and standard groups respectively. In severe case the 43.6% are decreased or revascularization respectively. This result is shown as short-term study of LMWH in unstable angina treatment and high dose treatment are required for > 6 days. Although the aspirin are given to the patients and the control groups did not receive UFH. In most countries this the standard protocol for the treatment. Another study FRIC was then performed.[8] 1482 patients are used for the study of diseases or non-Q-Wave infarction. The dose for LMWH are in randomized open design are 120 anti-Xa units/kg daily two times by continuous infusion for six days. In double blind i.e. in second phase the patients are assigned to LMWH was continued at dose of 7500IU once daily or placebo. The safety and efficacy for the treatment are equivalent to as same of pre- clinical trials. The 7.6% in UFH groups and 9.3% in LMWH groups are death outcomes of the composite of myocardial or recurrent angina occurs at 6th days. The rate of the composite end point of death for myocardial infraction are 3.6% and 3.9% respectively. In both groups the 12.3% are the death rate in myocardial infarction at days between 6 and 45. In both groups have very low bleeding. The result of the study is hypothesis that LMWH more effective as normal heparin. However, in placebo it's not more effective in administered of LMWH at 7500 U of dose as subcutaneously after 6 days, finding in the result of FRISC study. The 3171 patients are used in third study i.e. ESSENCE trial with disease or non-Q-wave myocardial infarction. The dose is 1 mg/kg (100anti Xa IU) SC of LMWH for every 12hrs or UFH administered as an I.V. bolus for 2 to 8 days with continuous infusion. Here the patients are randomized with double blind study. For two groups the 2.6 days are median for the treatment duration. In 14 days, in the treatment the patients are with diseases namely end point death, myocardial infraction is reduced or recurrent angina with LMWH. In UFH group 19.8% in LMWH 16.5% and 23.3% in UFH groups and 19.8% in LMWH groups are composite end point for 30 days which shows significant change in difference. The LMWH accounted to be low incidence in case of myocardial infraction, recurrent angina and even though there is no change in reduction in death or myocardial infraction. At 30 days there was no major bleeding difference (6.5% with LMWH vs 7.0% with UFH). In case of bruising at injection sites the bleeding was high in LMWH groups (18.4% vs 14.2%).

2.1. LMWH as a Nano-delivery system

Nano-delivery systems are the most explored novel drug delivery approaches, because of their ability to improve drug bioavailability, solubility and retention time owing to their small size, surface structure and high surface area.[9] from wide range of polymers, lipids, surfactants, dentrimers or combinations are colloidal carriers for the fabrication of a nano particle for localized therapeutic activity for weeks in addition to their penetration capability through various biological barriers. Lot of difficulty are faced in a nanotechnology platform like scaling-up difficulty, low-drug loading capacity, wide size distribution. Many researches are able to find different routes of administration of drugs as nano-carrier and it shows the significant progress the pegylated and conventional liposomes long lived encapsulating ardeparin (LMWH) are prepared in the nano size range (104.8 and 113nm, respectively) by hydration method.[10,11] They were successfully tested for their pharmacological efficacy models in rodent of PE and DVT following pulmonary administration. A once-every 48hrs inhaled dose showed similar therapeutic efficacy to a once-daily SC dosing regimen without any significant toxicity to lung tissues. Pegylation helped in avoiding the reduced half-life and bioavailability seen after three repeated dosing of the conventional liposomes. Dendrimer micelles present a promising class of nanocarriers that tested for pulmonary delivery of LMWHs. There is no positive effects on enoxaparin (LMWH) bioavailability in which the negatively charged dendrimers are occurs, the positively charged pegylated and non-pegylated dendrimers enhanced the relative bioavailability of the drug by 60.6 and 41.3%, respectively. At dose of 100 U/Kg administered drug at 48 hrs interval the pegylated formulation are given and its shows very close efficacy to 24 hrs SC administered drugs at dose of 50U/Kg in rats.

The bio adhesive is common which is prepared in production nanoparticle loaded with LMWH is chitosan and its derivatives[12–14] the bioavailability did not exceed 9% in these studies as per the[14] Bagre et al.[15] compared the oral bioavailability of chitosan nanoparticle. The alginate was used before and after coating to use for the overcome the solubility problem in chitosan. To improve the oral absorption the PH value should be more than 6.5 found in major parts of gastro intestinal tract to dissolve the prepared chitosan- based nanoparticle. A 1.6-fold higher oral bioavailability was attained by the coated nanoparticles compared to uncoated ones although no prolongation in the drug's half-life was observed. The hydroxy propyl

methyl cellulose phthalate (HPMCP) as enteric coated materials and thiolate chitosan as mucoadhesive are used to formulate enoxaparin (LMWH) loaded nanoparticles which possessing both PH -responsive and mucoadhesive properties polymer.[16] A pronounced improvement of oral BAV, which reached 21%, was reported holding promise for these systems as potential carriers for oral delivery of LMWHs.

2.2. *LMW Heparin from marine sources*

LMW Heparin from Natural Sources Bivalves Green lipped mussels found in waters off New Zealand were found useful in arthritis to gain better mobility in joints. The extracts of mussels have an anti-inflammatory effect.[17] This extract has been used safely like other conventional drugs without any side effects. At the Victoria Infirmary, Glasgow, 76% of the rheumatoid and 45% of the osteoarthritis patients reported improvement with the use of mussel extract. In India, marine mollusks and clams were examined for the recovery of heparin. Sulphated polysaccharide as a chemical present in heparin is reported to arrest bleeding from ruptured blood vessels and is also useful in the treatment of atherosclerosis. Presently, heparin is prepared from animal tissues that are rich in mast cells, such as porcine intestinal mucosa and beef lung. Naturally occurring low molecular weight heparin may be harvested more cheaply source from India's abundant coastal waters. The anticoagulant heparin with 3-o-sulphated glycosamine residues contain antithrombin dependent anticoagulant activity in mollusk.[18] Although the mollusk heparin contains antithrombin activity more than commercial heparin. So, this can be used in conversion of LMW Heparin for DVT, Myocardial infarction etc using heparinase enzyme in enzymatic hydrolysis process. This investigation focuses on the extraction, partial purification by fractionation and depolymerization of the heparin to yield LMWH from bivalve mollusk Katelysia opima, are present more amount in India especially in south India. After extraction, heparin was removed and depolymerized by nitrous acid and fractionated through DEAE cellulose column. Both forms of heparins were analyzed for their properties. *Coelomactra antiquata* mollusk species are having the anticoagulant activity. APTT, PT, and TT were commonly used to evaluate the anticoagulant activity of samples in medicine. The coagulation pathways with endogenous represent the APTT, PT is exogenous pathway and TT was plasma conversion of fibrinogen to fibrin.[19] APTT are concentration dependent pathway in G15. In the mammalian heparins (HP) there is slightly lower the prolongation effects of G15 and three times higher of G15 when given 500 μg/mL of mammalian heparins. In TT the same manner the G15 was increased by increasing the dose of 40 μg/mL as this results in anticoagulant effects through the endogenous pathways and exogenous pathway and also the reduce the stoppage of conversion of fibrinogen to fibrin and in Chinese pharmacopoeia the G15 was observed as 149.63 IU/mg The 33 IU/mg and 95 IU /mg were observed as anticoagulant in titers of crab and shrimp heparins respectively. The previous paragraph same as they have the anticoagulant effects by endogenous pathway and stoppage of conversion of fibrinogen to fibrin.[20,21] Due to presence of high sulfated content 21.44% and presence of N,3,6-trisulfated glucosamine the shrimp heparin and crab heparin have high content of anticoagulant and more action pathways.[22]

Marine organisms are remaining a largely unexploited reservoir, many of which contain a biotechnology/pharmaceutical application. Many organisms which is composed of molecules, materials and exhibiting the already present in current use. This an additional value added for the products which derived from the natural sources development of novel medical oriented when it is compared with the existing product as standard. Similarly, the terrestrial source, marine organisms, contain biomolecules with polysaccharides in different section and along with this the other similar to polysaccharides like hexose, hexosamine also present. The above polysaccharides are synthesized with the enzyme heparinase for the yield of LMW Heparin from natural sources.[23] The 73.5% in bovine mucosal heparin and 72.8% porcine mucosal heparin polysaccharide present is low when compared to marine mollusk.[24] The polysaccharides are hexoses with polymer composed of carbohydrates with link in glycosidic bonds. In another case all polysaccharide is similar the only difference is differed from them is the properties of polymers. The most of the bioactive compounds are reported to similar in structure, biomolecules mechanism of action molecular effects in on cell receptors and also estimated by pre-clinical studies using the rodents of animals with promising outcomes.

The main advantage of LMW Heparin in marine sources are low risk in the microorganism's contamination because of the terrestrial mammals. As a result, the drugs from this source are highly active in mammalian cells to overcome the diseases like anticoagulation. In alternative point in marine sources the heparin prepared are should be acceptable with scientifically and finically for the LMW Heparin preparation and also the yield obtained from this source are reasonably when compared with other methods already in existing in the market and used in the manufacturing of pharmaceutical heparin. In different part of world effectively refined for long time in use of LMW Heparin from several species like marine mollusks and sea cucumbers. Marine sources especially the aqua culture technologies were ton quantities are used as raw materials for the production of LMW Heparin. The sea cucumber reaches 35,000 tons in 2019 and the marine scallops reaches 95,000 tons in the world production of heparin. Possibly, the polysaccharide present in marine sources are more found examinations of their

properties on mammalian systems of their mechanism are compared with the standard one and from these few sources are in clinical trials stage of 3-4.[25]

2.3. Enzymatic degradation of shrimp heparin Penaeus brasiliensis

The heparinase are responsible for the product obtained from the shrimp heparin and this is prepared by heparin fragments or heparin 100mg with 0.1U of enzymes was incubated with ethylenediamine-acetate as 0.05M buffer, PH 7 for 8 hrs at 30^3C in a Nacl volume of 20 Wl. The incubation mixtures were then spotted in Whatman No.1 paper and subjected to chromatography in isobutyric acid: 1 M NH3, 5/3, v/v or isobutyric acid:1.25 M NH3, 5/3.6, v/v for 48 h. The unsaturated products formed were identified by short wave UV lamp and silver nitrate staining. The incubation mixtures were also analysed by HPLC in a 0.95U25 cm SAX ion exchange resin (Dupont) and the disaccharides formed were columned and eluted by using an NaCl gradient (0.01^2 M) with a £ow of 1 ml/ min and monitored at 230 nm. For large scale preparation of the fragments, 5 mg of heparin was incubated with 5 U of heparinase with ethylenediamine acetate as 0.05M buffer at PH 7 for 8 hrs at 300C in a Nacl volume of 500 Wl. The mixture was then fractionated by molecular sieving in a GS- 320 HPLC column (1U20 cm, exclusive MW 40 kDa) coupled to a GS-220 HPLC column (1U20 cm, exclusive MW 3 kDa) (Asahipak GS series, Asahi Chemical Industry Co., Yakoo, Japan) previously equilibrated in 0.8 M CaCl2. The eluted formed products are from the column with a £ow of 1 ml/min using 0.8 M CaCl2. At 230nm the unsaturated products were monitored and at 200nm the ungraded heparin was monitored to remove calcium chloride the fraction with each peak was combined, dried and suspended in 10 ml of acetone. With acetone the obtained precipitate washed twice and dried. For calibration of HPLC Columns the unfractured heparin molecular weight 15,000 Daltons, low molecular weight heparin 6000 Daltons and ULMW 35000 Daltons with hexa, tetra and disaccharides are used.

2.4. Preparation of LMW heparin by liquid phase plasma Mmethod

The pulsed electric discharge was generated by a needle-to-needle electrode geometry system in a double annular tube type reactor. In heparin aqueous solution directly electrical generated with discharged electrical plasma in high frequency power supply of bipolar pulse were used. The expectation of reason behind them are by symmetrical plasma generation comes from pulse bipolar system and to confirm with stable operation methods by the tip of the both electrodes cleaned by the products at either side of cathode or anode were done. The applied voltage, pulse width, and 250 V, 5μs frequency and 30 kHz, respectively. The Heparin were purchased from sigma-aldrich corporation (Grade I-A, =180 USP units/mg) which is prepared from porcine intestinal mucosa. Double distilled water was used in the study to make use a solution for the irradiation purpose about 0.5mg/ml of concentration of heparin are used to circulate about 300ml of solution in the reactor at a flow rate of 300 cc/min. The obtained LMWH were observed by FT-IR (FT- IR, Nicolet iS10, Thermofisher), Nuclear magnetic resonance (NMR, AVANCE 400, Bruker), and size exclusion chromatography/multi-angle laser light scattering for average molecular weight analysis.

2.5. Preparation of LMW heparin by chemically modified fractions

When compare to standard heparin the preliminary experiments are more resistance to nitrous acid than the natural heparin. This method under the reaction conditions it reacted with nitrous acid and increase the modified reaction time. The PH of the solution is adjusted to 3.5 by addition of 0.5M Hcl where the standard heparin (60mg) in water (1ml) was added with NaNo2 (30mg). Then the solution is neutralized with 0.1M NaoH and kept for 60min at 450C. NaBH4 (16mg) was added and the mixture was kept for 90min at 20^0c, to remove more borohydride the PH were adjusted at 3 addition of M Hcl then to 7 by 0.5M NaoH. The column (2.0×95cm) of sephadex G-75 was used, the neutralized solution prepared in 0.2M NH4HCO3 is applied to the column and followed by elution with the same solvent. The solvent collected at flow rate of 40ml/min and 2.5 ml fraction, to this uranic acid assay was done as per the method of Bitter and Muir With chondroitin sulfate standard the elution obtained are calibrated and A530 total segments are divided of > 12 000, 12 000, 9 300, 6 000, 4 300, and < 4 300. Freeze dried the sample which is obtained by each segment corresponding to standard LMW Heparin. The natural LMW Heparin corresponding fractions are purified by nitrous acid (pH 3.5 for 30 min at 4^0C) according to the procedure of Perlin et al.

2.6. Chromatography separation of LMWH

The physical or chemical properties of the individuals LMWH mainly depends on the chromatography technique for the isolation of crude or unfractionated heparin. There is no simple or straight forward method for the purification of LMWH, if so, the methods are inactivated. So, for the purification the selection of methods is very important. The process which is selected for the purification are done in several successive steps. The pharmacological application is not only used for the treatment. The main sequence is removal of contaminants (CS, HA & DS) and also the net yield is high for LMW Heparin. Generally, the gel filtration and ion-exchange chromatography are used for the separation of LMW Heparin with various

concentration of Nacl from marine mollusks.[26,27] The anticoagulant activity obtained from the marine mollusks through chromatography are compared with commercial one. In other cases, for separation into stationary phases the, SAX (strong anionic exchange resin) chromatography is also used for LMWH/HS.[28] There is no specific HPLC column for separation of the LMW Heparin because of the structural variations and Selecting dialysis membrane cutoff size also plays a major role in LMWH/HS separation from UFH. With crude 0.1-0.4 volumes of ethanol are precipitated by heparin in the mixture. Particularly at 0.3 and 0.4 fraction are rich with these components in GAG species. The precipitate with mixture volumes from 0.5 to 0.8 Has the, DS and CS are relative percentages. If the volume of ethanol is increased from 0.5to 0.8 DS amount is also is increased and decrease in CS and at 95% purity of DS can be obtained from 0.7-0.8 volume of ethanol. The CS of high purity precipitate is obtained by 1.0-2.0 volume of ethanol. To obtain high purity of heparin from GAG species the ethanol mixtures are used from 0.7 to 0.8 volumes of DS and 1.0 volumes used for CS. These sequence from getting the heparin from bovine lungs purification the CS are used with ethanol.[29] In olden day in carbohydrates chemistry practical method are used to separate different carbohydrates by relative solubility improvement. In LMWH/HS some precipitation alteration of fraction was done with the solvent namely addition of salts, distilled water, organic solvents, altering PH and temperature. This precipitation fraction is commonly used for obtaining the impurities and separation of nucleic acids.

3. Conclusion

The above review was discussed about its preparation and importance of low molecular weight heparin. The LMWH are used to kill many animals for preparing the heparin and from the heparin the LMWH are prepared and an alternate source should be finding to make more use of heparin from them in order to avoid the killing of the animals. So, in future the more clinical trials are concentrated in use of natural source of heparin especially from marine source. The heparin basically has sulphated polysaccharides; this polysaccharide is more richly available from marine sources especially from marine algae.

4. Source of Funding

None.

5. Conflict of Interest

None.

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